VOLUME V NUMBER 3

JOURNAL OF BACTERIOLOGY

OFFICIAL ORGAN OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

MAY, 1920

EDITOR-IN-CHIEF
C.-E. A. WINSLOW

It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR.

PUBLISHED BIMONTHLY
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON: E. C.

Entered as second-class matter April 17, 1918, at the postoffice at Baltimore, Maryland, under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 16, 1918.

Copyrighted, 1920, Williams and Wilkins Company

Price, net postpaid { $5.00 per volume, United States, Mexico, Cuba
$5.25 per volume, Canada
$5.50 per volume, other countries

GASTRON

The new Fairchild organic extract of the actual tissue juice of the entire stomach mucosa.

The power which Gastron exerts in the stomach is that exerted by the gastric juice; the service which Gastron renders is derived from the fact that it presents the whole complex of stomach gland principles—organic and inorganic, activating, digestive, antiseptic.

Indicated in disorders of gastric function; a useful accessory, compatible with, and contributing to, the success of many therapeutic measures—by maintaining digestion and nutrition, and promoting toleration of remedies.

Gastron is a gratefully agreeable solution, alcohol free.

Usual dose: one to two teaspoonfuls.

FAIRCHILD BROS. & FOSTER
NEW YORK

NUTROSE

We are now prepared to accept orders for small quantities of NUTROSE for immediate delivery. This material is satisfactory for the preparation of bacteriological culture media.

Nutrose has been unobtainable for some time and as our production is still limited, future requirements should be estimated in advance.

This is a typical example of our ability to furnish materials that are difficult to secure.

Write for our new 432 page catalog

Established 26 years

Chicago **ARTHUR DAIGGER & Co.** Illinois

THE FAMILIES AND GENERA OF THE BACTERIA

FINAL REPORT OF THE COMMITTEE OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS ON CHARACTERIZATION AND CLASSIFICATION OF BACTERIAL TYPES

C.-E. A. WINSLOW, *Chairman*, JEAN BROADHURST, R. E. BUCHANAN, CHARLES KRUMWIEDE JR., L. A. ROGERS, AND G. H. SMITH[1]

I. INTRODUCTION

The first report of the Committee on Characterization and Classification of Bacterial Types was published in the Journal of Bacteriology for September, 1917, vol. 2, p. 505, and was disdiscussed in some detail at the succeeding meeting of the Society.

In this preliminary report the committee reviewed briefly the historical development of systematic bacteriology from Ehrenberg to Orla-Jensen, discussed the principles of botanical nomenclature with extensive citations from the International Rules of Botanical Nomenclature, and presented a tentative system of classification of the Schizomycetes into families and genera.

The detailed classification presented was criticised in certain respects in the full discussion which followed, and in later correspondence between the committee and other members of the Society, and it was felt desirable that the scheme presented in 1917 should be revised in certain particulars and made more definite and if possible supplemented by an index of genera showing where the commoner bacterial species should be placed.

It was found impossible to complete this task and to prepare a list of approved genera for the 1918 meeting of the Society, but the task set before the committee has been at last completed and the committee is ready to make its final report at this time.

[1] Prof. R. S. Breed has coöperated actively in the work of the committee during the past two years, but has not felt that he could accept formal membership on the committee.

In discussions which have taken place between the members of the committee during the past two years the first question to be decided was whether the committee should simply present a list of approved genera for adoption by the Society, or should also prepare a revision of the general scheme of classification presented in 1917.

It was felt by some that the presentation by the committee of any scheme of classification would tend to give such a scheme undue authority and to impose arbitrary limits upon the development of the changing science of systematic bacteriology. It should be recognized most clearly that the limits of biological groups must always be subject to change with the growth of knowledge. The classification presented at this time seems to the committee the most reasonable outline for true biological relations among the bacteria which can be drawn up in the state of present knowledge, but this outline will necessarily be modified with the progress of investigations by individual systematists of the future. It is exceedingly improbable that any member of the committee would present the same classification in 1925 that is presented today. Indeed as to the position of certain genera the committee is itself in serious doubt at the present time.

In spite of these facts the Committee was of the opinion that it would be helpful to the members of the Society of American Bacteriologists to have the best judgment of the members of the committee as to the most natural method of classification at present available, particularly in view of the desirability of correcting certain errors in the earlier report already in type. Your committee has therefore prepared a modified arrangement of the families and genera of the Actinomycetales and Eubacteriales, which is presented as section III of this report. The sequence of families and genera has no special significance, and in some cases it is doubtful in which families certain genera should be placed. The 38 genera themselves that are here presented are however believed by the committee to represent for the most part real biological groups.

The second general problem, which had to be met by the committee, concerned the method of defining bacterial genera. A

recent report of the committee on generic types of the Botanical Society of America, published in Science for April 4, 1919, has urged that the application of generic names should be determined by type species rather than by attempts at generic characterization; and with this point of view the members of the committee on characterization and classification of bacterial types are in accord. The committee was agreed that type species with proper literature references should be included in all the genera listed, but that it was also desirable to include brief characterizations of the genera themselves. The situation with which we deal in attempting to classify the bacteria is somewhat different from that which exists among the higher plants. In the latter case actual type species have been deposited in herbaria and are available for reference; while among the bacteria this is not the case except for a few type species which have recently been deposited in the collection of the American Museum of Natural History in New York. In consideration of the uncertainty which surrounds the description of many bacterial species it was felt that it would be helpful to furnish at least tentative characterizations of the genera presented, and this policy has therefore been pursued in the preparation of section III of this report.

It will perhaps be convenient to indicate briefly the general changes in classification which distinguish the present report from that of 1917. The main departures are as follows:

The group recognized in 1917 as the family Mycobacteriaceae has now been elevated to the rank of an order Actinomycetales, and divided into two families, Actinomycetaceae and Mycobacteriaceae. To the former family we have added the genera *Actinobacillus* and *Erysipelothrix*, and we have omitted *Nocardia*, which Breed (1919) has recently shown should be combined for the present with *Actinomyces*. To the second family we have added the genus *Pfeifferella*.

The Nitrobacteriaceae have been divided into two tribes, the Nitrobactereae and Azotobactereae, and the definition of the family has been modified to permit the inclusion of *Rhizobium* which recent investigations have shown to possess peritrichous flagella, but whose general characteristics ally it clearly with

Azotobacter. The name *Acetobacter* has been substituted for *Mycoderma* to characterize the vinegar organisms.

Among the Coccaceae a new tribe was created for the genus *Neisseria*. The genus *Albococcus* is united with *Staphylococcus* and the new genera *Diplococcus* and *Leuconostoc* are added.

The Bacteriaceae are divided into seven tribes: Chromobactereae, Erwineae, Bactereae, Lactobacilleae, Pasteurelleae, Hemophileae, and Zopfeae, and the new genera *Erythrobacillus*, *Chromobacterium*, *Zopfius*, and *Proteus* are added. The Lactobacillaceae, originally recognized as a distinct family, are thus classed as a tribe of the Bacteriaceae.

For the convenience, particularly of students, we have prepared in section IV of this report an artificial key to the families and genera of the Actinomycetales and the Eubacteriales, which we hope may be of value. It should be possible to place a key of this kind in the hands of a student and enable him at least to determine the general generic group to which any organism belongs. In the case of certain genera the specific types can be easily identified by reference to monographs such as those of Wenner and Rettger (1919) on Proteus, Ford (1916) on Bacillus, Winslow, Rothberg and Parsons (1920) on Staphylococcus, and Winslow, Kligler and Rothberg (1919) on Bacterium.

Finally in section V of this report we have presented a generic index of the commoner species of bacteria with the names ordinarily used in the texts and with the new nomenclature indicated by the proposed classification. This list has been prepared by Miss Dorothy F. Holland of the Department of Public Health of the Yale School of Medicine. It is not intended to be exhaustive or to deal in any sense with problems of *specific* identity, but merely to serve as an index of generic reference for the more familiar types.

II. SPECIFIC RECOMMENDATIONS

The classification presented by the committee, the key and the generic index, as stated above, represent merely the consensus of opinion of the members of the committee as corresponding to the most natural system of classification indicated by present knowl-

edge. They are in no sense presented as official or binding. On the other hand in order that stability of nomenclature may be assured it is essential that certain generic names should be formally adopted by the Society, and where necessary established in the future by an International Botanical Congress, as genera conservanda. Such genera are provided for in the International Rules of Botanical Nomenclature in cases where a strict application of the rules of nomenclature, and especially the principle of priority starting from a certain date, would produce confusing and disadvantageous changes. In our own case it seems desirable to preserve in this way a number of generic names which have come into such general use that their abandonment would cause confusion, particularly in dealing with the large number of medical bacteriologists who are not familiar with the principles of botanical taxonomy. It is essential to proceed in a somewhat conservative fashion if any influence for good is to be exerted upon general practice in this field.

The following names are recommended for adoption as approved genera:

Acetobacter Fuhrmann	Leuconostoc Van Tieghem
Actinomyces Harz	Micrococcus Cohn
Bacillus Cohn	Rhizobium Frank
Bacterium Ehrenberg	Sarcina Goodsir
Chromobacterium Bergonzini	Spirillum Ehrenberg
Clostridium Prazmowski	Staphylococcus Rosenbach
Erythrobacillus Fortineau	Streptococcus Rosenbach
Leptotrichia Trevisan	Vibrio Mueller

Its work so far as possible being completed, we recommend that the Committee on Characterization and Classification of Bacterial Types be discharged and that a new Committee on Bacterial Taxonomy be appointed (1) to study and report to the Society from time to time in regard to problems of nomenclature, including such revisions of the nomenclature in the present report as may seem necessary; and (2) to take the proper steps to secure action at the next International Botanical Congress leading to the general ends contemplated in the 1916 recommendations of the Society:

(a) That French, English or German may be substituted for Latin in the diagnosis of bacterial species.

(b) That the date of publication of the third edition of Zopf's Spaltpilze (1883) be considered the beginning of bacterial nomenclature for the purpose of determining priority, with the exception of a definite list of genera conservanda.

(c) That such of the approved generic names specified above as may be found to require such action be recognized as genera conservanda in bacterial taxonomy.[2]

III. SUGGESTED OUTLINE OF BACTERIAL CLASSIFICATION

THE CLASS SCHIZOMYCETES

Minute, one-celled, chlorophyll-free, colorless, rarely violet-red or green-colored plants, which typically multiply by dividing in one, two or three directions of space. The cells thus formed are usually spherical, cylindrical, comma-shaped, spiral or filamen tous and are often united into filamentous, flat, or cubical aggregates. Filamentous species often surrounded by a common sheath. The cell plasma generally homogeneous without a morphologically differentiated nucleus. Reproduction by simple fission. In many species resting bodies are produced, either endospores or gonidia. Cells may be motile by means of flagella.

A. ORDER MYXOBACTERIALES[3]

Cells united during the vegetative stage into a pseudoplasmodium which passes over into a highly-developed cyst-producing resting stage.

B. ORDER THIOBACTERIALES[3]

Cells free or united in elongated filaments. Typically water forms, not cultivable on ordinary media. Life energy derived mainly from oxidative processes. Cells typically containing either granules of free sulphur or bacterio-purpurin or both, usually growing best in the presence of hydrogen sulphide.

[2] The Society of American Bacteriologists took favorable action on these resolutions at its meeting December 29, 1919.

[3] These first three orders are included briefly to give the complete setting of the fourth and fifth with which we are primarily concerned.

C. ORDER CHLAMYDOBACTERIALES[3]

Cells normally united in elongated filaments, often showing false but never true branching. Typically water forms. Sulphur and bacterio-purpurin are absent. Iron often present and usually a well-marked sheath.

D. ORDER ACTINOMYCETALES Buchanan 1917a, p. 162

Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. No pseudo-plasmodium. No deposits of free sulphur or iron. No bacteriopurpurin. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Some species are parasitic in animals or plants. Not water forms. Complex proteins frequently required. As a rule strongly aerobic, (except for some species of *Actinomyces* and the genera *Fusiformis* and *Leptotrichia*) and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

Family 1. Actinomycetaceae Buchanan 1918a, p. 403

Filamentous forms often branched and sometimes forming mycelia. Conidia sometimes present. Some species parasitic.

Genus 1. *Actinobacillus* Brumpt, 1900, p. 849

Filament formation, resembling streptobacilli. In lesions no mycelium formed, but at peripheries finger shaped branched cells are visible. Gram negative. Not acid fast.

Type species, *Act. Lignieresi* Brumpt.

Genus 2. *Leptotrichia* Trevisan 1879, p. 138

Synonyms: Leptothrix Robin 1847, not *Leptothrix* Keutzing 1843; not *Leptothrix* Zopf 1885; *Rasmussenia* Trevisan 1889.

Thick, long, straight or curved threads, unbranched, frequently clubbed at one end and tapering to the other. Gram positive when young. Threads fragment into short, thick rods. Anaerobic or facultative. Non-motile. Filaments sometimes granular. No aerial hyphae or conidia. Parasites or facultative parasites.

The type species is *Leptotrichia buccalis* (Robin 1847) Trevisan.

Genus 3. Actinomyces Harz 1877, p. 125

Synonyms: Streptothrix Cohn 1875, not *Streptothrix* Corda 1839; *Discòmyces* Rivolta 1879; *Nocardia*, Trevisan 1889; *Micromyces* Gruber 1891, not *Micromyces* Dangeard 1888; *Oospora* Sauvageau and Radais 1892; not *Oospora* Wallroth 1833; *Thermoactinomyces* Tsilinsky 1899; *Cohnistreptothrix* Pinoy 1913.

Organism growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Sometimes parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. Some species are microaerophilic or anaerobic. Non-motile.

The type species is *Actinomyces bovis* Harz.

Genus 4. *Erysipelothrix* Rosenbach, 1909, p. 367

Rod-shaped organisms with a tendency to the formation of long filaments which may show branching. The filaments may also thicken and show characteristic granules. No spores. Non-motile. Gram-positive. Do not produce acid. Microaerophilic. Usually parasitic.

The type species is *Erysipelothrix rhusiopathiae* (*Bacillus rhusiopathiae suis* Kitt 1893; *Mycobacterium rhusiopathiae* Chester 1901; *Erysipelothrix porci* Rosenbach 1909), the causal organism of swine erysipelas.

Family II. Mycobacteriaceae Chester 1897, p. 63

Parasitic forms. Rod shaped, frequently irregular in form but rarely filamentous and with only slight and occasional branching. Often stain unevenly (showing variations in staining reaction within the cell). No conidia.

Genus 1. *Mycobacterium* Lehmann and Neumann, 1896a, p. 363

Synonyms: Coccothrix Lutz 1886; *Sclerothrix* Metschnikoff 1888, not *Sclerothrix* Kuetzing 1849; *Mycomonas* Jensen 1909.

Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched cells. Non-motile, Gram-positive. No endospores. Growth on media slow. Aerobic. Several species pathogenic to animals.

The type species is *Mycobacterium tuberculosis* (Koch 1882) Lehmann and Neumann.

Genus 2. *Corynebacterium* Lehmann and Neumann 1896b, p. 350

Synonyms: Corynemonas Jensen 1909; *Corynethrix* Bongert 1901.

Slender, often slightly curved, rods with tendency to club and pointed forms, branching cells reported in old cultures. Barred uneven staining. Not acid fast. Gram-positive. Non-motile. Aerobic. No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

The type species is *Corynebacterium diphtheriae* (Loeffler 1884) Lehmann and Neumann.

Genus 3. *Fusiformis* Hoelling 1910, p. 240

Synonym: Mantegazzaea Vuillemin 1913, not *Mantegazzaea* Trevisan 1879.

Obligate parasites. Anaerobic or microaerophilic. Cells frequently elongate and fusiform, staining somewhat unevenly. Filaments sometimes formed; non-branching. Non-motile. No spores. Growth in laboratory media feeble.

The type species is *Fusiformis termitidis* Hoelling.

Non-fusiform types like *B. acne* and the anaerobic types found in Hodgkin's disease may for the present be tentatively left in this genus.

Genus 4. *Pfeifferella* Buchanan 1918b, p. 54

Non-motile rods, slender, Gram-negative, staining poorly, sometimes forming threads and showing a tendency toward branching. Gelatin may be slowly liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

Type species, *Pfeifferella mallei* (Loeffler 1886) Buchanan (the glanders bacillus)

The real lines of demarcation between the genera *Actinobacillus*, *Erysipelothrix*, *Fusiformis* and *Pfeifferella* and their relations to *Actinomyces* on the one hand and to *Mycobacterium* on the other seem very obscure and the above arrangement can be considered as only tentative.

E. ORDER EUBACTERIALES Buchanan 1917b, p. 162

The order Eubacteriales includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with hydrogen sulphide or other sulphur compounds, the cells in consequence containing neither sulphur granules nor bacterio-purpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. These organisms are usually minute and spherical, rod-shaped or spiral, in most genera not producing true filaments; and rarely branching. The cells may occur singly, in chains or other groupings. They may be motile by means of flagella, or non-motile; but are never *notably* flexuous. Cell multiplication occurs always by transverse, never by longitudinal fission. Some genera produce endospores, particularly the rod-shaped types. Conidia not observed. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudoplasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the *Myxobacteriales*.

Family I. NITROBACTERIACEAE Buchanan, 1917c, p. 349

Organisms usually rod-shaped (sometimes nearly spherical in *Nitrosomonas* and possibly in *Azotobacter*.) Cells motile or non-motile. Branched involution forms in *Rhizobium* and *Aceto-*

bacter. Endospores never formed. Obligate aerobes, capable of securing growth energy by the direct oxidation of carbon, hydrogen or nitrogen or of simple compounds of these. Non-parasitic (except in genus Rhizobium)—usually water or earth forms.

Tribe I. Nitrobactereae

Organisms deriving their life energy from oxidation of simple compounds of carbon and nitrogen (or of alcohol).

Genus 1. *Hydrogenomonas* Orla-Jensen 1909, p. 311

Monotrichic short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water). Kaserer (1905) who first described the organism states that his species will also grow well on a variety of organic substances.

The type species is *Hydrogenomonas pantotropha* (Kaserer 1906) Orla-Jensen. Nikleuski (1910) described two additional species, *H. vitrea* and *H. flava*.

Genus 2. *Methanomonas* Ora-Jensen 1909, p. 311

Monotrichic short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxide and water). The type species is *Methanomonas methanica* (Söhngen 1906) Orla-Jensen.

Genus 3. *Carboxydomonas* Orla-Jensen 1909, p. 311

Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxide (forming carbon dioxide). The type species, *Carboxydomonas oligocarbophila* (Beijerinck and van Delden 1903) Orla-Jensen, is described as non-motile.

Genus 4. *Acetobacter* Fuhrmann 1905, p. 8

Synonyms: Mycoderma Persoon 1822; *Ulvina* Kuetzing 1837; *Umbina* Naegeli 1849; *Bacteriopsis?* Trevisan 1885; *Gliacoccus* Maggi 1886; *Acetimonas* Jensen 1909.

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions as obligate aerobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells may occur as involution forms.

The type species is *Acetobacter aceti* (Thomson 1852), Committee.

Genus 5. *Nitrosomonas* Winogradsky 1892a, p. 127

Includes *Nitrosococcus* Winogradsky 1892

Cells rod-shaped or spherical, motile or non-motile, if motile with polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrites. Growth on media containing organic substances scanty or absent.

The type species is *Nitrosomonas europoea* Winogradsky.

Genus 6. Nitrobacter Winogradsky 1892b, p. 87

Synonym: Nitrosobacterium? Rullmann 1897.

Cells rod-shaped, non-motile, not growing readily on organic media or in the presence of ammonia. Cells capable of securing growth energy by the oxidation of nitrites to nitrates.

The type species is *Nitrobacter Winogradskyi*, Committee 1917a, p. 552.

Tribe 2. AZOTOBACTEREAE

Nitrogen-fixing organisms

Genus 7. *Azotobacter* Beijerinck 1901a, p. 561

Synonyms: Parachromatium Beijerinck 1903; *Azotomonas* Jensen 1909.

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when

motile, with tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen.

The type species is *Azotobacter chroococcum* Beijerinck.

Genus 8. *Rhizobium* Frank, 1889, p. 338.

Synonyms: Phytomyxa Schroeter 1886; *Cladochytrium* Vuillemin 1888; *Rhizobacterium* Kirchner 1895; *Pseudorhizobium* Hartleb 1900; *Rhizomonas* Jensen 1909.

Comment. *Phytomyxa* Schroeter has priority over *Rhizobium*, but because of the confusion which would arise from the substitution of the older correct name for the better known term *Rhizobium*, the committee recommends the adoption of the latter.

Minute rods, motile when young. Involution forms abundant and characteristic when grown under suitable conditions. Obligate aerobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates in the absence of compounds of nitrogen. Produce nodules upon the roots of leguminous plants.

The type species is *Rhizobium leguminosarum* Frank.

Family II. Pseudomonadaceae, Committee 1917b, p. 555

Rod-shaped, short, usually motile by means of polar flagella or rarely non-motile. Aerobic and facultative. Frequently gelatin liquefiers and active ammonifiers. No endospores. Gram stain variable, though usually negative. Fermentation of carbohydrates as a rule not active. Frequently produce a water-soluble pigment which diffuses through the medium as green, blue, purple, brown, etc. In some cases a non-diffusible yellow pigment is formed. Many yellow species are plant parasites.

Genus 1. *Pseudomonas* Migula 1894, p. 237, emended

Synonyms: Bacterium Ehrenberg emended Cohn 1872; *Bactrillum* Fischer 1895; *Arthrobactrinium* Fischer 1895; *Arthrobactrillum* Fischer 1895; *Eupseudomonas* Migula 1895; *Bactrinius* Kendall 1902; *Bactril-*

lius Kendall 1902; *Bacterium* Ehrenberg emended E. F. Smith 1905; *Denitromonas* Jensen 1909; *Liquidomonas* Jensen 1909.

Characters, those of family.

Type species, *Ps. aeruginosa* (Schroeter) Frost?

FAMILY III. SPIRILLACEAE. Migula 1894, p. 237

Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

Genus 1. *Vibrio* Mueller 1786, p. 39, emended E. F. Smith 1905

Synonyms: Pacinia Trevisan 1885; *Microspira* Schroeter 1886; *Pseudospira* De Toni and Trevisan 1889; *Liquidovibrio* Jensen 1909; *Solidovibrio* Jensen 1909; *Photobacterium?* Beijerinck 1889.

Cells short bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aerobic and anaerobic. No endospores. Usually Gram-negative. Water forms, a few parasites.

The type species is *Vibrio comma* (Koch 1884) Schroeter 1886.

Genus 2. *Spirillum* Ehrenberg 1830, p. 38 emended Migula 1894, p. 237

Synonyms: Spirobacillus? Metschnikoff 1889; *Spirosoma* Migula 1894; *Sporospirillum?* Jensen 1909.

Cells, rigid rods of various thicknesses, length, and pitch of the spiral, forming either long screws or portions of a turn. Usually motile by means of a tuft of polar flagella (5 to 20) which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles; their number varies greatly and is difficult to determine; since in stained preparations several are often united into a common strand. Endospore formation has been reported in some species. Habitat: water or putrid infusions.

Type species *Spirillum undula* (Mueller 1786) Ehrenberg.

FAMILY IV. COCCACEAE Zopf 1884, p. 45, emended Migula 1894

Synonyms: Sphaerobacteria Cohn 1872; *Coccaceen* Zopf 1884; *Coccogenae* Trevisan 1885; *Coccacei* Schroeter 1886; *Coccobacteria* Schroeter 1886; *Sphaerobacteries* Maggi 1886; *Kokkaceen* Hueppe 1886; *Coccacees* Mace 1897.

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division they are frequently flattened in the plane of division, and form chains, packets or irregular masses. Motility rare. Endospores absent. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates. Pigment often produced.

Tribe A. NEISSEREAE, Nov. Trib.

Strict parasites, failing to grow or growing very poorly on artificial media. Cells normally in pairs. Gram-negative. Growth fairly abundant on serum media.

Genus 1. *Neisseria* Trevisan 1885, p. 105

Synonyms: Diplococcus Weichselbaum 1886 in part; *Gonococcus?* Neisser? 1879; *Merismopedia* Zopf 1885; not *Merismopedia* Meyen 1839.

Characters, those of tribe.
Type species, *N. gonorrhoeae* Trevisan.

Tribe B. STREPTOCOCCEAE Trevisan, 1889a, p. 1051 emended

Parasites (thriving only or best on or in the animal body) except genus *Leuconostoc*. Grow well under anaerobic conditions. Many forms grow with difficulty on serum-free media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid but no gas in glucose and generally in lactose broth. Pigment, if any, white or orange.

Genus 2. *Diplococcus* Weichselbaum 1886, p. 506 emended

Synonyms: Klebsiella Trevisan 1885, in part; *Hyalococcus* Schroeter 1886; *Pseudodiplococcus* Bonome, 1888; *Pneumococcus?* Schmidlechner 1905.

Parasites, growing poorly, or not at all, on artificial media. Cells usually in pairs of somewhat elongated cells, often capsulated, sometimes in chains. Gram positive. Fermentative powers high, most strains forming acid in glucose, lactose, sucrose and inulin.

Type species, *D. pneumoniae* Weichselbaum.

Genus 3. *Leuconostoc* Van Tieghem 1878, p. 198, emended

Synonyms: Ascococcus Cienkowski 1878; not *Ascococcus* Cohn 1875; *Leucocystis?* Schroeter 1886.

Saprophytes, usually growing in cane sugar solutions. Cells in chains or pairs, united in large zoogleal masses. Some types at least Gram negative.

Type species, *L. mesenteroides* (Cienkowski) Van Tieghem.

Genus 4. *Streptococcus* Rosenbach 1884a, p. 22, emended Winslow and Rogers 1905, p. 669

Synonyms: Sphaerococcus Marpmann 1885, not *Sphaerococcus* Agardh 1821; *Arthrostreptokokkus* Hueppe 1886; *Perroncitoa* Trevisan 1889; *Babesia?* Trevisan 1889; *Schuetzia* Trevisan 1889; *Lactococcus* Beijerinck 1901; *Hypnococcus* Bettencourt et al. 1904; *Myxokokkus* Gonnermann 1907, not *Myxococcus* Thaxter 1892; *Melococcus?* Amiradzibi 1907; *Diplostreptococcus* Lingelsheim 1912

Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. Capsules rarely present, no zoogleal masses. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Many sugars fermented with formation of large amount of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates.

Type species is *Streptococcus pyogenes* Rosenbach.

Genus 5. *Staphylococcus* Rosenbach 1884b, p. 19

Synonyms: Micrococcus Cohn 1872 em. Migula 1894; *Botryomyces* Bollinger 1888; *Botryococcus* Kitt 1888, not *Botryococcus* Kuetzing 1849; *Galactococcus* Guillebeau; *Bollingera* Trevisan 1889; *Gaffkya* Trevisan 1885; *Pyococcus* Ludwig 1892; *Carphococcus* Hohl 1902; *Albococcus* Winslow and Rogers 1906; *Aurococcus* Winslow and Rogers 1906; *Indolococcus* Jensen 1909; *Liquidococcus* Jensen 1909; *Peptonococcus* Jensen 1909; *Enterococcus?* (Thiercelin) Rougentzoff 1914.

Parasites. Cells in groups and short chains, very rarely in packets. Generally stain by Gram. On agar streak good growth, of white or orange color. Glucose, maltose, sucrose and often lactose, fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

Type species is *Staphylococcus aureus* Rosenbach.

Tribe C. Micrococceae Trevisan, 1889b, p. 1067, emended (as Metacoccaceae) Winslow and Rogers 1905, p. 669

Facultative parasites or saprophytes. Thrive best under aerobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles; cell aggregates in groups, packets or zoogleal masses. Generally decolorize by Gram. Pigment yellow or red.

Genus 6. *Micrococcus* Cohn 1872 a, p. 153, emended Winslow and Rogers, 1905, p. 669

Synonyms: Microsphaera Cohn 1872, not *Microsphaera* Leveille 1851; *Ascococcus* Cohn, 1875; *Pediococcus* Balcke 1884; *Merista* Van Tieghem 1884, not *Merista* (Banks and Soland) Cunningham 1839; *Planococcus* Migula 1894; *Urococcus* Miquel 1879; not *Urococcus* Kuetzing 1849; *Carphococcus* Hohl 1902; *Pedioplana* Wolff 1907; *Tetradiplococcus?* Bartoszewicz and Schwarzwasser 1906; *Solidococcus* Jensen 1909; *Planomerista* Vuillemin 1913.

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). Generally decolorize by Gram. Growth on agar abundant, with formation

of yellow pigment. Glucose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

The type species is *Micrococcus luteus* (Schroeter) 1872b, Cohn.

Genus 7. *Sarcina* Goodsir 1842, p. 432, emended Winslow and Rogers 1905, p. 359

Synonyms: Urosarcina Miquel 1879; *Planosarcina* Migula 1894; *Pseudosarcina?* Maze 1903; *Tetradiplococcus?* Bartoszewicz and Schwarzwasser 1908; *Lactosarcina* Beijerinck 1908; *Sporosarcina?* Jensen 1909.

Sarcina differs from *Micrococcus* solely in the fact that cell division occurs under favorable conditions in three planes, forming regular packets.

The type species is *Sarcina ventriculi* Goodsir.

Genus 8. Rhodococcus Zopf 1891, p. 28, emended Winslow and Rogers 1906, p. 546

Synonyms: Not *Rhodococcus* Molisch 1907.

Saprophytes. Cells in groups or regular packets. Generally decolorize by Gram. Growth on agar abundant with formation of red pigment. Glucose broth slightly acid, lactose broth neutral. Gelatin rarely liquefied. Nitrates generally reduced.

Type species, *Rhodococcus rhodochrous* Zopf.

FAMILY V. BACTERIACEAE Cohn 1872b, p. 231
Emended Committee 1917c, p. 560

Rod-shaped cells without endospores. Usually Gram-negative. Flagella when present peritrichic. Metabolism complex, aminoacids being utilized, and generally carbohydrates.

Tribe 1. *Chromobactereae,* Nov. Trib.

Water bacteria producing a red or violet pigment.

Genus 1. *Erythrobacillus*, Fortineau 1905, p. 104

Synonyms: Zaogalactina Sette 1824; *Serratia* Bizio, 1825; *Bacillus*, in part, of many authors.

Small aerobic bacteria, producing a red or pink pigment, usually a lipochrome. Gram stain variable. It is possible that related yellow and orange chromogens should be included here as well.

Type species, *Erythrobacillus prodigiosus* (Ehrenberg) Committee.

Genus 2. *Chromobacterium* Bergonzini 1881, p. 153

Synonyms: The name was spelled *Cromobacterium* by Bergonzini and corrected by Zimmerman 1881.

Aerobic bacteria, producing a violet chromoparous pigment, soluble in alcohol but not in chloroform. Motility and Gram reaction variable.

Type species, *Chr. violaceum* Bergonzini.

Tribe 2. ERWINEAE, Nov. Trib.

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

Genus 3. *Erwinia* Committee 1917d, p. 560.

Characters those of the tribe.

Type species, *E. amylovora* (Burrill 1883, p. 319; Trevisan 1889, p. 19) Committee 1917.

Tribe 3. ZOPFEAE, Nov. Trib.

Gram positive rods, growing freely on artificial media. Not attacking carbohydrates.

Genus 4. *Zopfius*, Wenner and Rettger, 1919, p. 334

Synonyms: Bacterium Ehrenberg 1828 in part; *Bacillus* Cohn, 1872 in part; *Proteus* Hauser 1885, in part.

Long rods occurring in evenly curved chains. Gram positive. Motile. Proteus-like growth on media. Facultative anaerobes. Carbohydrates and gelatin not attacked, hydrogen sulphide not formed.

Type species, *Z. zopfii* (Kurth) Wenner and Rettger.

Tribe 4. Bactereae, Nov. Trib.

Gram negative rods growing freely on artificial media. Generally forming acid from carbohydrates and often gas composed of CO_2 and H_2.

Genus 5. *Proteus* Hauser 1885, p. 1

Synonyms: Spirulina Hueppe 1886; not *Spirulina* Turpin 1827; *Liquidobacterium* Jensen 1909.

Highly pleomorphic rods, filaments and curved cells being common as involution forms. Gram negative. Actively motile. Characteristic amoeboid colonies on moist media. Liquefy gelatin rapidly and produce vigorous decomposition of proteins. Ferment glucose and sucrose (but usually not lactose), with formation of acid and gas (the latter being CO_2 only).

Type species, *P. vulgaris* Hauser.

Genus 6. *Bacterium* Ehrenberg 1828, emended Orla-Jensen 1909, p. 315

Synonyms: Tyrothrix Duclaux 1879; *Actinobacter* Duclaux 1882 in part; *Klebsiella* Trevisan 1885 in part; *Kurthia?* Trevisan 1885; *Gliscrobacterium* Malerba and Sanna Salaris 1888; *Pneumobacillus?* Arloing 1889; *Aerobacter* Beijerinck 1900; *Salmonella* Lignieres 1900; *Pyobacillus* Koppanyi 1907.

Gram negative, evenly staining rods. Often motile, with peritrichic flagella. Easily cultivable, forming grape-vine leaf or convex whitish surface colonies. Liquefy gelatin rarely. All forms except *B. alcaligenes* and the *B. abortus* group attack the hexoses and most species ferment a large series of carbohydrates. Acid formed by all, gas (CO_2 and H_2) only by one series. Typi-

cally intestinal parasites of man and the higher animals although several species may occur on plants and one (*B. aerogenes*) is widely distributed in nature. Many species pathogenic.

Type species, *B. coli* Escherich 1885, p. 518.

Tribe 5. Lactobacilleae, Nov. Trib.

Rods, often long and slender, Gram-positive, non-motile, without endospores. Usually produce acid from carbohydrates, as a rule lactic. When gas is formed, it is CO_2 without H_2. The organisms are usually somewhat thermophilic. As a rule microaerophilic; surface growth on media poor.

Genus 7. *Lactobacillus* Beijerinck 1901b, p. 214

Synonyms: Dispora? Kern 1882; *Tyrothrix?* Duclaux 1882 in part; *Saccharobacillus?* van Laer 1889; *Lactobacter* Beijerinck 1901; *Streptobacillus* Rest and Khoury 1902; *Brachybacterium* Troili-Petersson 1903; *Caseobacterium* Jensen 1909.

Generic characters those of the tribe.

The type species is *Lactobacillus caucasicus* (Kern?) Beijerinck.

Tribe 6. *Pasteurelleae*, Nov. Trib.

Gram negative rods, showing bipolar staining. Parasitic forms of slight fermentative power.

Genus 8. *Pasteurella* Trevisan 1888, p. 7.

Synonyms: Octopsis? Trevisan 1885; *Coccobacillus* Gamaleia 1888, not *Coccobacillus* Leube 1885; *Dicoccia?* Trevisan 1889; *Diplobacillus?* Weichselbaum 1887.

Aerobic and facultative. Powers of carbohydrate fermentation slight; no gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals.

The type species is *Pasteurella cholerae-gallinarum* (Flügge 1886) Trevisan.

Tribe 7. Hemophilaeae, Nov. Trib.

Minute parasitic forms growing only in presence of hemoglobin, ascitic fluid or other body fluids.

Genus 9. *Hemophilus* Committee 1917c, p. 561

Synonyms: Pyobacillus? Koppanyi 1907; *Diplobacillus* Morex 1896, not *Diplobacillus* Weichselbaum 1887.

Minute rod-shaped cells, sometimes thread forming and pleomorphic, nonmotile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood serum or ascitic fluid. Gram negative.

The type species is *Hemophilus influenzae* (Pfeiffer 1893, p. 357) Committee 1917.

Family VII. Bacillaceae Fischer 1895, p. 139

Rods producing endospores, usually Gram-positive. Flagella when present peritrichic. Often decompose protein media actively through the agency of enzymes.

Genus 1. *Bacillus* Cohn, 1872c, p. 174

Synonyms: Bactrella? Morren 1830; *Metallacter?* Perty 1852; *Bacetridium* Davaine 1868 in part; *Urobacillus* Miquel 1879; *Pollendera* Trevisan 1884; *Zopfiella* Trevisan 1885; *Streptobacter* Schroeter 1886; *Cornilia* Trevisan 1889 in part; *Bacterium* Ehrenberg, emended Migula 1894 in part; *Bactridium* Fischer 1895, not *Bactridium* Wallroth 1832; *Bactrinium* Fischer 1895; *Bactrillum* Fischer 1895; *Endobacterium* Lehmann and Neumann 1896; *Astasia* Meyer 1898; *Fenobacter* Beijerinck 1900; *Bacterius* Kendall 1902 in part; *Aplanobacter* E. F. Smith 1905 in part; *Semiclostridium* Maassen 1905; *Plennbakertum* Gonnermann 1907; *Myxobacillus* Gonnermann 1907; *Thermobacillus* Jensen 1909; *Serratia* Vuillemin 1913 in part, not *Serratia* Bizio 1823.

Aerobic forms. Mostly saprophytes. Liquefy gelatin. Often occur in long threads and form rhizoid colonies. Form of rod usually not greatly changed at sporulation.

The type species is *Bacillus subtilis* Cohn.

Genus 2. *Clostridium* Prazmowski 1880, p. 23

Synonyms: Amylobacter Trecul 1865; *Cornilia* Trevisan 1889 in part; *Granulobacter* Beijerinck 1893; *Clostrillum* Fischer 1895; *Clostrinium* Fischer 1895; *Paracloster* Fischer 1895; *Semiclostridium* Maassen 1905 *Botulobacillus* Jensen 1909; *Butyribacillus* Jensen 1909; *Cellulobacillus* Jensen 1909; *Putribacillus* Jensen 1909.

Anaerobes or micro-aerophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* Prazmowski.

IV. ARTIFICIAL KEY TO THE FAMILIES AND GENERA OF THE ACTINOMYCETALES AND EUBACTERIALES

A. Typically filamentous forms. *Actinomycetaceae*
  B. Mycelium and conidia formed.....*Actinomyces*
  BB. No true mycelium
    C. Cells show branching
      D. Gram negative.....*Actinobacillus*
      DD. Gram positive.....*Erysipelothrix*
    CC. Cells never branch. Gram positive threads later fragmenting into rods.....*Leptotrichia*
AA. Typically unicellular forms (although chains of cells may occur)
  B. Spherical cells. *Coccaceae*
    C. Parasitic forms. Cells in pairs, chains or irregular groups, never in packets. Generally active fermenters
      D. Cells in flattened coffee-bean-like pairs Gram negative.....*Neisseria.*
      DD. Cells not as above. Gram positive
        E. Cells in lanceolate pairs or chains Growth on media not abundant
          F. Cells in lanceolate pairs. Inulin generally fermented..*Diplococcus*
          FF. Cells in chains. Inulin generally not fermented....*Streptococcus*
        EE. Cells in irregular groups. Growth on media fairly vigorous. White or orange pigment.....*Staphylococcus*
    CC. Saprophytic forms. Chains occurring in zoogleal masses in sugar solutions.....*Leuconostoc*
    CCC. Saprophytic forms. Cells in irregular groups or packets, not in chains. Fermentative powers low
      D. Packets formed.....*Sarcina*
      DD. No packets
        E. Yellow pigment.....*Micrococcus*
        EE. Red pigment.....*Rhodococcus*

BB. Rods
C. Curved rods. *Spirillaceae*
D. Short comma-like rods. One-three short flagella.....*Vibrio*
DD. Long spirals, five-twenty flagella.....*Spirillum*
CC. Straight rods
D. No endospores
E. Rods of irregular shape or showing branched or filamentous involution forms.
F. Animal parasites. Cells of irregular shape. Staining unevenly.
G. Acid fast.....*Mycobacterium*
GG. Not acid fast
H. Cells elongate, fusiform.....*Fusiformis*
HH. Cells not fusiform, sometimes branching
I. Gram positive. Slender, sometimes clubbed rods.....*Corynebacterium*
II. Gram negative. Rods sometimes form threads. Characteristic honey-like growth on potato..*Pfeifferella*
FF. Not animal parasites. Cells staining unevenly and with branched or filamentous forms at certain stages. Never acid fast.
G. Metabolism simple, growth processes involving oxidation of alcohol or fixation of atmospheric nitrogen (latter in symbiosis with green plants)
H. Cells minute, symbiotes in roots of leguminous plants.....*Rhizobium*
HH. Oxidizing alcohol, branching forms common....*Acetobacter*
GG. Not as above. Proteus-like colonies.
H. Not attacking carbohydrates. Gram +.....*Zopfius*
HH. Fermenting glucose and sucrose. Gram −.....*Proteus*
EE. Regularly formed rods
F. Metabolism simple, growth processes involving oxidation of carbon, hydrogen or their simple compounds or the fixation of atmospheric nitrogen. *Nitrobacteriaceae*
G. Fixing nitrogen or oxidizing its compounds
H. Fixing nitrogen
Cells large; in soil.....*Azotobacter*
HH. Oxidizing nitrogen compounds
I. Oxidizing ammonia.....*Nitrosomonas*
II. Oxidizing nitrites.....*Nitrobacter*
GG. Not as above
H. Oxidizing hydrogen.....*Hydrogenomonas*
HH. Not as above, using simpler carbon compounds
I. Oxidizing CO.....*Carboxydomonas*
II. Oxidizing CH_4.....*Methanomonas*
FF. Not as above
G. Flagella usually present, polar.
Pseudomonadaceae.....*Pseudomonas*
GG. Flagella when present peritrichic.
Bacteriaceae

H. Parasitic forms showing bipolar staining ..... *Pasteurella*
HH. Not as above
I. Strict parasites growing only in presence of hemoglobin or ascitic fluid ..... *Hemophilus*
II. Not as above
J. Water forms producing red or violet pigment
K. Pigment red ..... *Erythrobacillus*
KK. Pigment violet ..... *Chromobacterium*
JJ. Not as above
K. Plant pathogens ..... *Erwinia*
KK. Not as above
L. Gram positive, forming large amount of acid from carbohydrates and sometimes CO_2 but no H_2 ..... *Lactobacillus*
LL. Gram negative, forming H_2 as well as CO_2 if gas is produced ..... *Bacterium*
DD. Endospores present, *Bacillaceae*.
E. Aerobes ..... *Bacillus*
EE. Anaerobes ..... *Clostridium*

V. GENERIC INDEX OF THE COMMONER FORMS OF BACTERIA

PREPARED FOR THE COMMITTEE BY DOROTHY F. HOLLAND, DEPARTMENT OF PUBLIC HEALTH, YALE SCHOOL OF MEDICINE

The following index is not intended to be exhaustive or to deal in any sense with problems of *specific* identity. The literature has not been comprehensively studied and no attempt has been made to arrive at conclusions in regard to the priority of specific names. The list is simply an index of reference to show how the *names* of *species* commonly found in the literature should be changed to correspond with the generic classification suggested by the committee. Many of the specific names quoted are known to be synonyms, and the list is therefore in no sense a check list of valid bacterial species. Our hope is that those who wish to use the committee classification can by reference to this list easily replace the older form of any specific name by the newer one which it should bear in accord with the generic arrangement presented above; and in particular that it will facilitate the breaking up of the absurdly incongruous aggregates massed together under the older names Bacillus and Bacterium.

Acetobacter aceti (Thomson)
xylinum (Brown)
Actinobacillus Lignieresi Brumpt
Actinomyces
albido-flavus Rossi-Doria
alboflavus Waksman & Curtis
albosporeus (Krainsky?) Waksman & Curtis
albus (Krainsky?) Waksman & Curtis
asteroides (Eppinger) Gasperini
aurantiacus (Rossi-Doria) Gasperini
aureus Waksman & Curtis
bobili Waksman & Curtis
bovis Harz
Californicus Waksman & Curtis
carneus (Rossi-Doria) Gasperini
chromogenus Gasperini
citreus (Krainsky?) Waksman & Curtis
diastaticus (Krainsky?) Waksman & Curtis
exfoliatus Waksman & Curtis
farcinicus (Trev. and de Toni) Gasperini
flavus Sanfelice
Foersteri (Cohn) Gasperini
fradii Waksman & Curtis
griseus (Krainsky?) Waksman & Curtis
Halstedii Waksman & Curtis
Hofmanni (Gruber) Gasperini
Isreali Kruse
invulnerabilis (Acosta-Grande-Rossi) Kruse
Krausei
lavendulae Waksman & Curtis
Lipmanii Waksman
madurae (Vincent) Leh. and Neu.

Actinomyces—*Continued.*
melanocyclus Krainsky
melanosporeus Krainsky
necrophorus Loeffler
pheochromogenus Conn
pleuricolor
Poolensis Taubenhaus
pulmonalis
Rosenbachii Kruse
roseus (Krainsky?) Waksman & Curtis
rubidaureus (Thiry) Lachner
rubrus Kruse
Rutgersensis Waksman & Curtis
scabies (Thaxter) Güssow
thermophilus Gilbert
verne Waksman & Curtis
violaceus (Rossi-Doria) Gasperini
violaceus-ruber Waksman & Curtis
violaceus-accsari Waksman & Curtis

Azotobacter
agile Beijerinck
Beijerinckii Lipman
chroococcum Beijerinck
Vinelandii Lipman
Woodstownii Lipman

Bacillus
abortivius (*see* Bact. abortivium)
abortus Bang[4] (*see* Bact. abortum)
abortus-equi (*see* Bact. abortum-equi)
acidi-lactici Grotenfelt (*see* Bact. acidi-lactici)
acidificans-longissimus (*see* Lactobacillus acidificans-longissimus)
acidophil-aerogenes Torrey-Rahe (*see* Lactobacillus acidophil-aerogenes)

[4] B. bronchisepticus, B. abortus, B. melitensis according to Evans (Further Studies on Bact. abortus and Related Bacteria) J. Infect. Dis., Vol. 22, No. 6, p. 580) are related morphologically, culturally, biochemically, and serologically. They are Gram negative, do not form spores, "do not attack the sugars nor the other commonly used fermentable test substances." These organisms should probably constitute a distinct new genus, but we have hesitated to add new generic names at this time without further study of our own.

Bacillus—*Continued.*
acidophilus Moro (*see* Lactobacillus acidophilus)
acnes (*see* Fusiformis acnes)
adhaerens Ford
aerogenes Escherich (*see* Bact. aerogenes)
aerogenes-capsulatus Welch & Nuttall (*see* Clostridium aerogenes-capsulatum)
aeruginosus Schröter (*see* Pseudomonas aeruginosa)
agri Ford
albolactus Migula
alcaligenes Petruschky (*see* Bact. alcaligenes)
amethystinus Eisenberg (*see* Chromobacterium amethystinum)
amylobacter van Tieghem (*see* Clostridium amylobacter)
amyloruber Hefferan (*see* Erythrobacillus amyloruber)
amylovorus (Burrill-Trev.) *see* Erwinia amylovora)
anaerogenes Lembke (*see* Bact. anaerogenes)
anthracis Koch-Cohn
anthracis-symptomatici Kruse (*see* Clostridium anthracis-symptomatici)
anthracoides Hueppe-Wood
aquatilis Tataroff (*see* Pseudomonas aquatilis)
arborescens Frankland (*see* Erythrobacillus arborescens)
astheniae Dawson (*see* Bact. astheniae)
asterosporus (Meyer) Migula
aterrimus Leh. and Neu.
avisepticus Kitt (*see* Pasteurella aviseptica)
bibulus McBeth & Scales (*see* Bacterium bibulum)
bifidus Tissier (*see* Lactobacillus bifidus)
botulinus van Ermengem (*see* Clostridium botulinum)

Bacillus—*Continued.*
bovisepticus Kruse (*see* Pasteurella boviseptica)
brevis Migula
bronchicanis Ferry (*see* Bact. bronchicanis)
bronchisepticus Ferry[4] (*see* Bact. bronchisepticum)
buccalis Robin (*see* Leptotrichia buccalis)
bulgaricus Massol (*see* Lactobacillus bulgaricus)
Bütschlii
butyricus Hueppe
butyricus Botkin (*see* Clostridium butyricum)
campestris Pammel (*see* Pseudomonas campestris)
capsulatus Sternberg (*see* Bact. capsulatum)
capsulatus-mucosus Fasching (*see* Bact. mucosum-capsulatum)
carotovorus Jones (*see* Erwinia carotovora)
caucasicus Flügge (*see* Lactobacillus caucasicus)
centrosporus Ford
cerasus Griffin (*see* Pseudomonas cerasa)
cereuleus Voges (*see* Pseudomonas cereulea)
cereus Frankland
chauvei Arloing-Cornevin-Thomas (*see* Clostridium chauvei)
cholerae Koch (*see* Vibrio cholerae)
cholerae-gallinarum Flügge (*see* Pasteurella cholerae-gallinarum)
cholerae-suis Salmon-Smith (*see* Bact. cholerae-suis)
circulans Jordan
citri Hasse (*see* Pseudomonas citri)
cloacae Jordan (*see* Bact. cloacae)
cohaerens Gottheil
coli Escherich (*see* Bact. coli)
coli-communior Durham (*see* Bact. coli communior)
coli-communis Escherich (*see* Bact. coli-communis)

Bacillus—*Continued.*
comma Koch (*see* Vibrio comma)
coscoroba Trétrop (*see* Bact. coscoroba)
cuniculicida (Gaffky) Flügge (*see* Pasteurella cuniculicida)
cyanogenes Flügge (*see* Pseudomonas cyanogenes)
cypripedii Hori (*see* Erwinia cypripedii)
cytaseus McBeth & Scales (*see* Bact. cytaseum)
Danysz (*see* Bact. Danysz)
Delbrücki (*see* Lactobacillus Delbrücki)
diphtheriae Klebs-Loeffler (*see* Corynebacterium diphtheriae)
dysenteriae Flexner (*see* Bact. dysenteriae)
dysenteriae Shiga (*see* Bact. dysenteriae or Bact. Shigae)
edematis Koch (*see* Clostridium edematis)
Ellenbachensis α Stutzer-Hartleb
enteritidis Gärtner (*see* Bact. enteritidis)
enteritidis-sporogenes Klein (*see* Clostridium enteritidis-sporogenes)
erysipelatos-suis (Löffler) Migula (*see* Erysipelothrix erysipelatos-suis)
erythrogenes Grotenfelt (*see* Erythrobacillus erythrogenes)
fecalis-alcaligenes Petruschky (*see* Bact. fecalis-alcaligenes)
feseri (Trev.) Kitt (*see* Clostridium feseri)
fimi McBeth & Scales (*see* Bact. fimi)
flavidus Morse (*see* Corynebacterium flavidum)
fluorescens liquefaciens Flügge (*see* Pseudomonas fluorescens)
Friedmanii (*see* Mycobacterium Friedmanii)
Frostii (*see* Pseudomonas Frostii)
fuchsinus Boekhout-de Vries (*see* Erythrobacillus fuchsinus)
fusiformis Gottheil

Bacillus—*Continued.*
fusiformis Veillon and Zuber? (*see* Fusiformis)
gallinarum Klein (*see* Bact. gallinarum)
globigii Migula
graveolens Meyer and Gottheil
Havaniensis Sternberg (*see* Erythrobacillus Havaniensis)
Hoagii Morse (*see* Corynebacterium Hoagii)
Hoffmanii Loeffler (*see* Corynebacterium Hoffmanii)
hyacinthi Wakker? (*see* Pseudomonas hyacinthi)
icteroides Sanarelli (*see* Bact. icteroides)
indicus Koch (*see* Erythrobacillus indicus)
influenzae Pfeiffer (*see* Hemophilus influenzae)
juglandis Pierce (*see* Pseudomonas juglandis)
Kiliensis Fischer and Breunig (*see* Erythrobacillus Kiliensis)
lachrymans Erw. Smith and Bryan (*see* Pseudomonas lachrymans)
lactici-acidi Grotenfelt (*see* Bact. acidi-lactici)
lactis Flügge
lactis-acidi Leichmann (*see* Lactobacillusl actis-acidi or Streptococcus lacticus)
(lactis) aerogenes Escherich (*see* Bact. aerogenes)
(lactis) erythrogenes Grotenfelt (*see* Erythrobacillus erythrogenes)
lactis-viscosus Adametz (*see* Bact. lactis-viscosus)
lacunatus Morax and Axenfeld (*see* Hemophilus lacunatus)
laterosporus Ford
lathyri Manns and Taubenhaus (*see* Erwinia lathyri)
leprae Hansen (*see* Mycobacterium leprae)
levans Lehmann-Wolffin (*see* Bact. levans)

Bacillus—*Continued.*
liodermos (Flügge) Leh. and Neu.
lividus Flüge and Proskauer (*see* Chromobacterium lividum)
mallei Loefler and Schütz (*see* Pfeifferella mallei)
malvacearum Erw. Smith (*see* Pseudomonas malvacearum)
medicaginis Sackett (*see* Pseudomonas medicaginis)
megatherium de Bary
melitensis (Bruce)[4] (*see* Bact. melitensis)
melonis Giddings (*see* Erwinia melonis)
mesentericus (Flügge) Migula
miniaceus Zimmermann (*see* Erythrobacillus miniaceus)
mirabilis Migula (*see* Proteus mirabilis)
mori Boyer and Lambert (*see* Pseudomonas mori)
mortiferus Harris (*see* Fusiformis mortiferus)
mucosus Zimmermann
murisepticus Flügge (*see* Bact. murisepticum)
murium Loeffler (*see* Bact. murium)
mycoides Flügge
mycoides-roseus Scholl (*see* Erythrobacillus mycoides-roseus)
neapolitanus Fraenkel (*see* Bact. neapolitanum)
niger Migula
of Achalme (*see* Clostridium Welchii)
of Boas-Oppler (*see* Lactobacillus bulgaricus)
of Bordet-Gengou (*see* Hemophilus pertussis)
of Ducrey (*see* genus Hemophilus)
of Gärtner (*see* Bact. enteritidis)
of Klebs-Loeffler (*see* Corynebacterium diphtheriae)
of Koch-Weeks (*see* genus Hemophilus)
of Morax-Axenfeld (*see* Hemophilus lacunatus)
of Morgan (*see* Bact. Morgani)

Bacillus—*Continued.*
of Schottmüller (*see* Bact. Schottmülleri)
of Shiga (*see* Bact. Shigae)
of Sternberg (*see* Bact. Sternbergii)
oleae (Arcangeli) Trev. (*see* Pseudomonas oleae)
oleraceae Harrison (*see* Erwinia oleraceae)
oligocarbophilus Beijerinck and van Delden (*see* Carboxydomonas oligocarbophila)
ozaenae (Abel) Leh. and Neu, (*see* Bact. ozaenae)
panis Migula
pantotrophus Kaserer (*see* Hydrogenomonas pantotropha)
paracoli Widal & Nobecourt (*see* Bact. paracoli)
paradysenteriae (*see* Bact. paradysenteriae)
paralyticans Ford-Robertson (*see* Corynebacterium paralyticans)
paratyphi Schottmüller (*see* Bact. paratyphi)
paratyphosus A Schottmüller (*see* Bact. paratyphosum A)
paratyphosus B Schottmüller (*see* Bact. paratyphosum B)
perfringens Veillon & Zuber (*see* Clostridium perfringens)
pertussis Bordet and Gengou (*see* Hemophilus pertussis)
pestis Kitasato and Yersin (*see* Pasteurella pestis)
pestis-caviae (*see* Pasteurella pestis-caviae)
petasites Gottheil
phaseoli Smith (*see* Pseudomonas phaseoli)
phlegmones-emphysematosae Fraenkel (*see* Clostridium phlegmones-emphysematosae)
phosphorescens Fischer (*see* Vibrio phosphorescens)
phytophthorus Appel (*see* Erwinia phytophthora

Bacillus—*Continued.*
plicatus Frankland (*see* Pseudomonas plicata)
Plymouthensis Fischer (*see* Erythrobacillus Plymouthensis)
pneumoniae Friedländer (*see* Bact. pneumoniae)
Prausnitzii Trevisan
prodigiosus (Ehrenberg) (*see* Erythrobacillus prodigiosus)
proteus-fluorescens Jaeger (*see* Pseudomonas protea-fluorescens)
proteus-mirabilis (Hauser) (*see* Proteus mirabilis)
proteus-vulgaris (Hauser) (*see* Proteus vulgaris)
proteus-Zenkeri (Hauser) (*see* Zopfius Zenkeri)
pruni Erw. Smith (*see* Pseudomonas pruni)
pseudo-anthracis Burri
pseudodiphtheriae Loeffler (*see* Corynebacterium pseudodiphtheriae)
pseudo-tetanicus (Kruse) Migula
psittacosis Nocard (*see* Bact. psittacosis)
pullorum Rettger (*see* Bact. pullorum)
putrificus Flügge (*see* Clostridium putrificum)
pyocyaneus Gessard (*see* Pseudomonas pyocyanea)
pyogenes-foetidus Passet (*see* Bact. pyogenes-foetidum)
radicicola Beijerinck (*see* Rhizobium radicicola)
ramosus Frankland
rhinoscleromatis v. Frisch (*see* Bact. rhinoscleromatis)
rosaceus Migula (*see* Erythrobacillus rosaceus)
ruber Miquel (*see* Erythrobacillus ruber)
ruber Zimmermann (*see* Erythrobacillus ruber)
rubricus Hefferan (*see* Erythrobacillus rubricus)
ruminatus Gottheil

Bacillus—*Continued.*
rutilescens Hefferan (see Erythrobacillus rutilescens)
rutilus Hefferan (*see* Erythrobacillus rutilus)
salmoneus Dyar (*see* Erythrobacillus salmoneus)
sanguinarium Moore (*see* Bact. sanguinarium)
Savastanoi Erw. Smith (*see* Pseudomonas Savastanoi)
segmentosus (*see* Corynebacterium segmentosum)
Shigae Chester (*see* Bact. Shigae)
simplex Gottheil
smegmatis Alvarez-Tavel (*see* Mycobacterium smegmatis)
solanacearum Erw. Smith (*see* Erwinia solanacearum)
solanisaprus Harrison (*see* Erwinia solanisapra)
sporogenes Klein (*see* Clostridium sporogenes)
Sternbergii (*see* Bact. Sternbergii)
Stewarti Erw. Smith (*see* Pseudomonas Stewarti)
subtilis Cohn
subtilis-viscosus Chester
subviscorum Migula (*see* Bact. subviscorum)
suipestifer Kruse (*see* Bact. suipestifer)
suisepticus Kruse (*see* Pasteurella suiseptica)
synxanthus Ehrenberg (*see* Pseudomonas synxantha)
terminalis Migula
tetani Nicolaier (*see* Clostridium tetani)
tracheiphilus Erw. Smith (*see* Erwinia tracheiphila)
tuberculosis Koch (*see* Mycobacterium tuberculosis)
tumefaciens Erw. Smith and Townsend (*see* Pseudomonas tumefaciens)
tumescens Zopf
typhi (*see* Bact. typhi)

Bacillus—*Continued.*
typhi-exanthematici Plotz (*see* Fusiformis typhi-exanthematici)
typhi-murium Loeffler (*see* Bact. typhi-murium)
typhi-suis (*see* Bact. typhi-suis)
typhosus Eberth-Gaffky (*see* Bact. typhosum)
vascularum Cobb-Erw. Smith (*see* Pseudomonas vascularum)
violaceus (Schröter) Migula (*see* Pseudomonas violacea)
vulgaris (Hauser) (*see* Proteus vulgaris)
vulgatus (Flügge) Trevisan
Welchii Migula (*see* Clostridium Welchii)
x Sternberg (*see* Bact. Sternbergii)
xerosis Kuschbert-Neisser (*see* Corynebacterium xerosis)
xylinus Brown (*see* Acetobacter xylinum)
Zenkeri (Hauser) (*see* Zopfius Zenkeri)
Zopfii Kurth (*see* Zopfius Zopfii)

Bacterium abortivium
abortum (Bang)[4]
abortum-equi
acidi-lactici (Grotenfelt)
aërogenes (Escherich)
alcaligenes (Petruschky)
anaerogenes (Lembke)
angulatum Fromme (*see* Pseudomonas angulata)
astheniae (Dawson)
bibulum (McBeth & Scales)
bronchicanis
bronchisepticum (Ferry)[4]
campestris Pammel (*see* Pseudomonas campestris)
capsulatum (Sternberg)
casei α Orla-Jensen (*see* Lactobacillus casei)
casei ε Orla-Jensen (*see* Lactobacillus helveticus)
cholerae-suis (Salmon-Smith)
cloacae (Jordan)

Bacterium—*Continued.*
coli (Escherich)
coli-communior Durham
coli-communis Escherich
communior (Durham)
coscoroba (Trétrop)
Danysz
dysenteriae (Flexner)
dysenteriae (Shiga)
enteritidis (Gaertner)
fecalis-alcaligenes Petruschky
fimi (McBeth & Scales)
gallinarum (Klein)
hyacinthi Wakker (*see* Pseudomonas hyacinthi)
icteroides (Sanarelli)
lactis acidi (Leichmann) (*see* Lactobacillus lactis acidi or Streptococcus lacticus)
lactis viscosus
lepisepticum Ferry (*see* Pasteurella lepiseptica)
levans (Lehmann-Wolffin)
melitensis (Bruce)[4]
Morgani (Winslow-Rottenberg-Parsons)
mucosum capsulatum (Fasching)
murisepticum (Flügge)
neapolitanum (Fraenkel)
ozaenae [(Abel) Leh. and Neu.]
paracoli (Widal & Nobecourt)
paradysenteriae
paratyphi
paratyphosum A (Schottmüller)
paratyphosum B (Schottmüller)
pestis (Kitasato and Yersin) (*see* Pasteurella pestis)
phaseoli Erw. Smith (*see* Pseudomonas phaseoli)
psittacosis (Nocard)
pullorum (Rettger)
putidum Flügge (*see* Pseudomonas fluorescens var. non liquefaciens)
pyogenes foetidum (Passet)
rhinoscleromatis (v. Frisch)
sanguinarium (Moore)
Savastanoi Erw. Smith (*see* Pseudomonas Savastanoi)

Bacterium—*Continued.*
Schottmülleri (Winslow-Rottenberg-Parsons)
Shigae (Winslow-Rottenberg-Parsons)
Sternbergii
Stewarti Erw. Smith (*see* Pseudomonas Stewarti)
suipestifer (Kruse)
tularense McCoy and Chapin
typhi-exanthematici (Plotz) (*see* Fusiformis typhi-exanthematici)
typhi-murium (Loeffler)
typhi-suis
typhosum (Eberth-Gaffky)
vulgare (Hauser) (*see* Proteus vulgaris)
xylinum (Brown) (*see* Acetobacter xylinum)
Zopfii Kurth (*see* Zopfius Zopfii)

Betacoccus arabinosaceus Orla-Jensen (*see* Leuconostoc arabinosaceus)
bovis Orla-Jensen (*see* Leuconostoc bovis)

Carboxydomonas
oligocarbophila Beijerinck and van Delden

Chromobacterium
amethystinum-(Eisenberg)
janthinum (Zopf)
lividum (Flügge-Proskauer)
violaceum Bergonzini

Clostridium
aerogenes-capsulatum (Welch & Nuttall)
amylobacter (van Tieghem)
anthracis-symptomatici (Kruse)
botulinum (van Ermengem)
butyricum (Botkin)
butyricum Prazmowski
chauvei (Arloing-Cornevin-Thomas)
edematis (Koch)
enteritidis sporogenes
feseri (Trev.-Kitt)
pasteurianium (Winogradsky)
perfringens

Clostridium—*Continued.*
phlegmones emphysematosae (Fraenkel)
putrificum (Flügge)
sporogenes Klein
tetani (Nicolaier)
Welchii (Migula)

Corynebacterium
diphtheriae (Klebs-Loeffler) Leh. and Neu.
Hoagii (Morse)
Hoffmanii (Loeffler-Hoffman-Wellenhoff)
segmentosum
xerosis (Kuschbert-Neisser)

Diplococcus flavus Flügge (*see* Micrococcus flavus)
gonorrheae Neissser (*see* Neisseria gonorrheae)
intracellularis-meningitidis Weichselbaum (*see* Neisseria intracellularis-meningitidis)
involutus Kurth
lanceolatus Foa-Bordoni-Uffreduzzi
mucosus (Schottmüller)
pneumoniae Weichselbaum
Weichselbaumii (*see* Neisseria Weichselbaumii)

Erwinia
amylovora (Burrill-Trev.) Committee 1917
aroideae (Townsend)
carotovora (Jones)
lathyri (Manns and Taubenhaus)
melonis (Giddings)
oleraceae (Harrison)
phytophthora (Appel)
solanacearum (Erw. Smith)
solanisapra (Harrison)
teutlia (Metcalf)
tracheiphila (Erw. Smith)

Erysipelothrix
erysipelatos-suis
rhusiopathiae Kitt

Erythrobacillus
amyloruber (Hefferan)
erythrogenes (Grotenfelt)[5]
fuchsinus (Boekhout & de Vries)
havaniensis (Sternberg)
indicus (Koch)
Kiliensis (Fischer & Breunig)
(lactis) erythrogenes (Grotenfelt)[5]
miniaceus (Zimmermann)
mycoides-roseus (Scholl)
Plymouthensis (Fischer)
prodigiosus (Ehrenberg)
rubefaciens (Zimmermann)
ruber (Miquel)
ruber (Zimmermann)
rubricus (Hefferan)
rufus (Hefferan)
rutilescens (Hefferan)[5]
rutilus (Hefferan)

Fusiformis acnes
Hodgkini
termitidis (Hoelling)
typhi-exanthematici (Plotz)

Hemophilus of Ducrey[6]
influenzae (Pfeiffer)
of Koch-Weeks[6]
lacunatus (Morax-Axenfeld)
pertussis (Bordet-Gengou)

Hydrogenomonas
pantotropha Kaserer

Lactobacillus
acidificans-longissimus
acidophil-aerogenes (Torrey-Rahe)
acidophilus (Moro)
bifidus (Tissier)
bulgaricus (Massol)
casei (Orla-Jensen)

Lactobacillus—*Continued.*
caucasicus Flügge
cereale (Orla-Jensen)
Delbrücki
helveticus (Orla-Jensen)
jugurt (Orla-Jensen)
lactis (Orla-Jensen)
lactis-acidi Leichmann
planticus (Orla-Jensen)

Leptotrichia buccalis Robin

Leuconostoc arabinosaceus (Orla-Jensen)
bovis (Orla-Jensen)
mesenteroides (Cienkowski) van Tieghem

Methanomonas methanica Söhngen

Micrococcus acne (*see* Staphylococcus acne)
agilis Ali-Cohen (*see* Rhodococcus agilis)
candicans Flügge (*see* Staphylococcus candicans)
candidus Cohn (*see* Staphylococcus candidus)
casei
catarrhalis Pfeiffer (*see* Neisseria catarrhalis)
cinnabareus Flügge (*see* Rhodococcus cinnabareus)
citreus Dyar
citreus Passet
flavus (Flügge) Migula
gonorrheae (Trevisan) (*see* Neisseria gonorrheae)
intracellularis Weichselbaum (*see* Neisseria intracellularis)
lanceolatus Foa-Bordoni-Uffreduzzi (*see* Diplococcus)

[5] These organisms contain a water-soluble red pigment in contrast to the lipochrome of the organisms of the true prodigiosus group. They are placed here provisionally, but may call for separate generic classification.

[6] The bacillus of Ducrey, and of Koch-Weeks have not been given specific names. They seen to belong to this genus and are placed here awaiting specific names which would be less unwieldly than a genitive form of Ducrey and the combination Koch-Weeks.

Micrococcus—*Continued.*
liquefaciens
luteus (Schroeter) Cöhn
mastitidis
melitensis Bruce (*see* Bact. Melitensis
meningitidis Weichselbaum (*see* Neisseria meningitidis)
mycodermatus
mollis Dyar (*see* Staphylococcus mollis)
nigrofaciens Northrup
ochraceus Rosenthal
pharyngis-siccus Lingelsheim (*see* Neisseria pharyngis-sicci)
rheumaticus Poynton & Paine (*see* Streptococcus rheumaticus)
rhodochrous Zopf (*see* Rhodococcus rhodochrous)
rosaceus Frankland (*see* Rhodococcus rosaceus)
roseus Flügge (*see* Rhodococcus roseus)
ruber = M. (tetragenus) ruber Bujwid (*see* Rhodococcus ruber)
tetragenus Gaffky (*see* Staphylococcus tetragenus)
ureae Cohn Flügge (*see* Staphylococcus ureae)
varians Dyar
zymogenes (*see* Streptococcus gracilis)

Mycobacterium
Friedmanii
leprae Hansen
Moelleri
phlei (Moeller) Leh. & Neu.
rhusiopathiae Chester (*see* Erysipelothrix rhusiopathiae)
smegmatis Alvarez-Tavel
tuberculosis (Koch) Leh. & Neu.

Neisseria
catarrhalis (Pfeiffer)
gonorrheae Trevisan
intracellularis Weichselbaum
intracellularis-meningitidis Weichselbaum

Neisseria—*Continued.*
meningitidis (Weichselbaum)
pharyngis-sicci (Lingelsheim)
Weichselbaumii

Nitrobacter Winogradskyi

Nitrosomonas europaea-Winogradsky
javaniensis Winogradsky

Pasteurella
aviseptica (Kitt)
boviseptica (Kruse)
cholerae-gallinarum (Flügge) Trevisan
cuniculicida (Gaffky-Flügge)
lepiseptica (Ferry)
pestis (Kitasato-Yersin)
suiseptica (Kruse)

Pfeifferella mallei (Loeffler) Buchanan

Proteus
fluorescens Jaeger (*see* Pseudomonas protea fluorescens)
mirabilis Hauser
vulgaris Hauser
Zenkeri Hauser (*see* Zopfius Zenkeri)

Pseudomonas
aeruginosa (Schroeter) Frost?
angulata (Fromme)
aquatilis (Tataroff)
beticola (Smith)
campestris (Pammel)
cerasa (Griffin)
cereulea (Voges)
citri (Hasse)
cyanogenes
fluorescens (Flügge) Migula
Frostii
hyacinthi (Wakker?)
juglandis (Pierce)
lachrymans (Erw. Smith and Bryan)
malvacearum (Erw. Smith)
medicaginis (Sackett)
mori (Boyer-Lambert)
oleae (Arcangeli-Trev.)
phaseoli (Erw. Smith)
pisi Sackett

Pseudomonas—*Continued.*
protea Frost
protea-fluorescens (Jaeger)
pruni (Erw. Smith)
pyocyanea Gessard
Savastanoi (Erw. Smith)
Stewarti (Erw. Smith)
syncyanea Ehrenberg
synxantha (Ehrenberg) Cohn
tumefaciens (Erw. Smith and Townsend)
vascularum (Cobb-Erw. Smith)
viridilivida Brown

Rhizobium leguminosarum Frank
radicicola (Beijerinck)

Rhodococcus agilis (Ali-Cohen)
cinnabareus (Flügge)
fulvus Cohn
incarnatus Gruber
rhodochrous Zopf
rosaceus (Frankland)
roseus (Flügge)
ruber (Bujwid)

Sarcina
aurantiaca (Schröter-Cohn)
flava De Bary
lutea Schröter
rosea Schröter (*see* Rhod. roseus)
subflava Ravenel
ventriculi Goodsir

Spirillum
cholerae-asiaticae Zopf (*see* Vibrio cholerae-asiaticae)
concentricum Kitasato
danubicum Heider (*see* Vibrio danubicus)
desulfuricans Beijerinck (*see* Vibrio desulfuricans)
Ghinda Kruse (*see* Vibrio Ghinda)
Massowah Pasquale-Pfeiffer (*see* Vibrio Massowah)
Metchnikovi Gamaleia (*see* Vibrio Metchnikovi)
Milleri (*see* Vibrio Milleri)
of Deneke (*see* Vibrio tyrogenus)

Spirillum—*Continued.*
of Finkler-Prior (*see* Vibrio Finkleri)
phosphorescens Fischer (*see* Vibrio phosphorescens)
rubrum v. Esmarch
serpens Müller-Zettnow
tyrogenum Deneke (*see* Vibrio tyrogenus)
undula Ehrenberg
volutans Ehrenberg

Staphylococcus
acne
albus
aurantiacus-Schröter-Cohn (*see* Sarcina aurantiaca)
aureus Rosenbach
candicans (Flügge)
candidus
canescens Migula
cereus-albus Passet
cereus-flavus Passet
epidermidis
mollis (Dyar)
pyogenes albus Rosenbach
pyogenes-aureus Rosenbach
tetragenus (Gaffky)
ureae (Cohn-Flügge)

Streptobacterium casei (*see* Lactobacillus casei)

Streptobacterium plantarum (*see* Lactobacillus plantarum)

Streptococcus
bovis Orla-Jensen
conglomeratus Kurth
cremoris Orla-Jensen
endocarditis
epidemicus Davis
erysipelatos Fehleisen
faecium Orla-Jensen
glycerinaceus Orla-Jensen
gracilis Escherich
hemolyticus Rolly
inulinaceus Orla-Jensen
lacticus Kruse
lacticus-mastitidis Orla-Jensen

Streptococcus—*Continued.*
lactis Orla-Jensen
liquefaciens Orla-Jensen
mucosus Schottmüller (*see* Diplococcus mucosus)
pneumoniae Weichselbaum (*see* Diplococcus pneumoniae)
pyogenes Rosenbach
rheumaticus (Poynton & Paine)
thermophilus Orla-Jensen
viridans Schottmüller
zymogenes

Tetracoccus liquefaciens (*see* Micrococcus liquefaciens)
casei (*see* Micrococcus casei)
mastitidis (*see* Micrococcus mastitidis)
mycodermatus (*see* Micrococcus mycodermatus)

Thermobacterium
cereale Orla-Jensen (*see* Lactobacillus cereale)
lactis Orla-Jensen (*see* Lactobacillus lactis)
bulgaricum Orla-Jensen (*see* Lactobacillus bulgaricus)

Thermobacterium—*Continued.*
helveticum Orla-Jensen (*see* Lactobacillus helveticus)
jugurt Orla-Jensen (*see* Lactobacillus jugurt)

Vibrio
aquatilis Günther
berolinensis Migula
cholerae-asiaticae (Zopf)
comma Schröter
danubicus (Heider)
desulfuricans (Beijerinck)
Dunbari
fetus Th. Smith
Finkleri (Schröter)
Ghindae (Kruse)
Massowah (Pasquale-Pfeiffer)
Metchnikovi (Gamaleia)
Milleri
phosphorescens (Fischer)
proteus Buchner
Schuylkilliensis Abbott
tyrogenus (Deneke)

Zopfius
Zenkeri (Hauser)
Zopfii (Kurth)

REFERENCES

This list includes all references to descriptions of accepted genera and type species. References to descriptions of synonyms will be found in the 1917 and 1918 papers by Buchanan listed below.

Beijerinck, M. W. 1901a Über oligonitrophile Mikroben. Centralbl. f. Bakt. II Abt., **7**, 561–582.

Beijerinck, M. W. 1901b Sur les ferments lactiques de l'industrie. Arch. Neerlandaises des Sciences Exact. et Nat., Ser. 2, **6**, 214.

Beijerinck, M. W., and van Delden, A. 1903 Über eine farblose Bakterie, deren Kohlenstoffnahrung aus der atmosphärischen Luft herrührt. Centralbl. f. Bakt. Abt. II, **10**, 33–47.

Bergonzini, Curzio 1881 Sopra un nuova Batterio Colorato. Annuario della Societa dei Naturalisti in Moderna. Ser. 2, **14**, 149–158.

Bizio, B. 1823 Polent. porporp. Bibl. ital., **30**, 288.

Breed, R. S., and Conn, H. J. 1919 Nomenclature of the Actinomycetaceae. J. Bact., **4**, 585.

Brumpt, E. 1910 Précis de Parasitologie. Paris.

BUCHANAN, R. E. 1917a, b Studies in the nomenclature and classification of the bacteria. II. The primary subdivisions of the Schizomycetes. J. Bact., **2**, 155–164.

BUCHANAN, R. E. 1917c Studies in the nomenclature and classification of the bacteria. III. The families of the Eubacteriales. J. Bact., **2**, 349.

BUCHANAN, R. E. 1918a Studies in the nomenclature and classification of the bacteria, VIII. The subgroups and genera of the Actinomycetales. J. Bact., **3**, 403.

BUCHANAN, R. E. 1918b, c Studies in the nomenclature and classification of the bacteria. V. Subgroups and genera of the Bacteriaceae. J. Bact., **3** (b) 54. (c) 51.

BURRILL, F. J. 1883 A new species of Micrococcus (bacteria). Am. Naturalist, **7**, 319.

CHESTER, F. D. 1897 Classification of the Schizomycetes. 9th Annual Report. Delaware College Agr. Exper. Sta., 62–63.

CHESTER, F. D. 1901 A manual of determinative bacteriology, p. 352.

COHN, FERD. 1872a Untersuchungen über Bakterien. I. Beitr. z. Biol. d. Pflanzen, **1**, 153 (Heft 1).

COHN, F. 1872b Organismen in der Pockenlymphe. Virchows Arch., **55**, 237.

COHN, F. 1872c Untersuchungen über Bakterien. II. Beiträge z. Biologie d. Pflanzen, **1**, (Heft 3), 141–208.

COMMITTEE ON CHARACTERIZATION AND CLASSIFICATION OF BACTERIAL TYPES: Society of American Bacteriologists, C. E. A. Winslow, Chairman 1917. Preliminary Report. The Families and Genera of the Bacteria. J. Bact. **2**, (a) 552, (b) 555, (c) (d) 560, (e) 561.

DE TONI, J. B. AND TREVISAN, V. 1889 Schizomycetaceae in Saccardo. Sylloge Fungorum, **8**, 923–1087.

EHRENBERG, C. G. 1828 Symbolae Physicae, animalia evertebrata.

EHRENBERG, C. G. 1830 Beiträge zur Kenntniss der Organisation der Infusorien und ihrer geographischen Verbreitung. Abhandlungen d. Berl. Akad., pp. 1–88.

ESCHERICH, TH. 1885 Die Darmbakterien des Neugeborenen u. Säuglinge. Fortschritte der Medizin, **3**, 1885, 515–522, and 547–554.

FISCHER, A. 1895 Untersuchungen über Bakterien. Jahrb. für Wissensch. Bot., **27**, Heft 1, 1–163.

FLÜGGE, C. 1886 Die Mikroorganismen. 2 Aufl.

FORD, W. W. 1916 Aerobic spore-bearing non-pathogenic bacteria. Classification. J. Bact., **1**, 527.

FORTINEAU, L. 1905 L'Erythrobacillus pyosepticus. Compt. rend. Soc. Biol. **58**, 104.

FRANK, B. 1889 Über die Pilzsymbiose der Leguminosen. Ber. d. deut. bot. Gesellsch., **7**, 338.

FUHRMANN, F. 1905 Morphologisch-biologische Untersuchungen über ein neues Essigsaures-bildendes Bakterium. Beiheft zum bot. Centralbl., **19**, 1–31.

GOODSIR, JOHN 1842 History of a case in which a fluid periodically ejected from the stomach contained vegetable organisms of an undescribed form. Edinburgh Med. & Surg. J., **57**, 432.

HARZ, C. O. 1877–78 *Actinomyces bovis*, ein neuer Schimmel in dem Gewebe des Rindes. Jahresber. d. Muenchener Central Thierarznei Schule, pp. 125–140.

HAUSER 1885 Fäulnisbakterien, p. 1.

HOELLING 1910 Die Kernverhältnisse von Fusiformis termitidis. Arch. f. Protistenkunde, **19**, 240.

KASERER, H. 1906 Die Oxidation des Wasserstoffes durch Mikroorganismen Centralbl. f. Bakr. II Abt, **16**, 681–696.

KITT 1893 Bakterienkunde u. path. Mikroskopie, p. 284.

KOCH, R. 1882 Die Aetiologie der Tuberkulose. Berl. klin. Wochenschrift, No. 15.

KOCH, R. 1884 Conferenz zur Erörterung der Cholerafrage. Berl. klin. Wochenschrift, No. 31, 477. Continued in No. 32.

LEHMANN, K. B., AND NEUMANN, R. 1896 Atlas und Grundriss der Bakteriologie. München (a) **2**, 363; (b) **2**, 350.

LOEFFLER 1884 Untersuchungen über die Bedeutung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe. Mitteilungen aus dem Kaiserl. Gesundheitsamt., **2**, 421.

LOEFFLER 1886 Die Aetiologie der Rotzkrankheit. Arbeiten aus dem Kais. Gesundheitsamt, **1**, 141.

MIGULA, W. 1894 Uber ein neues System der Bakterien. Arb. a. d. bakt. Instit. der technisch. Hochschule zu Karlsruhe, **1**, 235–238.

MUELLER, O. F. 1773 Vermium terrestrium et fluviatilium.

MUELLER, O. F. 1786 Animalcula infusoria, p. 39.

ORLA-JENSEN 1909 Die Hauptlinien des natürlichen Bakteriensystems. Centralbl. f. Bakt, II Abt., **22**, 305–346.

PASTEUR, L. 1868 Etude sur le Vinaigre. p. 106.

PERSOON 1822 Mycologia europeae. Sectio prima, p. 96.

PFEIFFER, R. 1893 Die Aetiologie der Influenza. Zeit. f. Hyg., **13**, 357–385.

PRAZMOWSKI, ADAM 1880 Untersuchungen über die Entwickelungsgeschichte und Fermentwirkung einiger Baterien, Leipzig, p. 23.

ROBIN 1847 Des végétaux qui croissent sur les animaux vivans. Paris, p. 42.

ROSENBACH 1884 Mikroorganismen bei den Wundinfektionskrankheiten des Menschen (a) p. 22; (b) p. 19.

ROSENBACH, F. J. 1909 Experimentelle, morphologische und klinische Studien über die Krankheitserregenden Mikroorganismen des Schweinerothlaufs, des Erysipeloides, und der Mäusesepsis. Ztschr. f. Hyg., **63**, 343–371.

SCHRÖTER, J. 1872a Über einige durch Bakterien gebildete Pigmente in Cohn's Beitrage zur Biologie, Bd. I, Heft 2, p. 126.

SCHRÖTER, J. 1872b Über einige durch Bakterien gebildete Pigmente. Beiträge z. Biol. d. Pflanzen. **1**, Heft 2, a. p. 119.

SCHRÖETER, J. 1886 Die Pilze Schlesiens. In Cohn, Kryptogamen-Flora von Schlesien, p. 168.

SMITH, E. F. 1905 Bacteria in relation to plant diseases, Vol. 1. Washington.

SÖHNGEN, N. L. 1906 Über Bakterien, welche Methan als Kohlenstoffnahrung und Energiequelle gebrauchen. Centralbl. f. Bakt. Abt. II, **15**, 513–517.

THOMSON, R. D. 1852 Über die Natur und die chemischen Wirkungen der Essigmutter. Annalen der Chemie und Pharmazie, **83**, 89.

TREVISAN, V. 1879 Prime linee d'introduzione allo studio dei Batterjitaliani. Rendiconti Reale Instituto Lombardo di Scienze e Lettere. IV, Series 2, 12, 133–151.

TREVISAN, V. 1885 Caratteridi alcuni nuovi generi di Batteriacee. Atti della Accademia Fisio-Medico-Statistica in Milano. Series, 4, 3, pp. 92–107.

TREVISAN, V. 1888 Sul micrococco della rabbia, p. 7.

TREVISAN, V. 1889 I generi e le specie delle batteriaceae, prodromo sinottico. Milano (a) 1051; (b) p. 1067.

VAN TIEGHEM 1878 Sur la gomme de sucrerie. Ann. d. Sc. nat. 6 Ser. Bot., **7**, 180–202.

WEICHSELBAUM, A. 1886 Untersuchungen über Pneumonie. Bakt. Theil. Med. Jahrb., 82 (Neue Folge 1), 506.

WENNER, J. J., AND RETTGER, L. F. 1919 A systematic study of the Proteus group of Bacteria. J. Bact., **4**, 331.

WINOGRADSKY, S. 1892 Contributions a la morphologie des organismes de la nitrification. Archives de sciences biologiques. St. Petersbourg, (a) T. I, No. 1, 2, p. 127, b. p. 87.

WINSLOW, C.-E. A., KLIGLER, I. J., AND ROTHBERG, WM. 1919 Studies on the classification of the colon-typhoid group of bacteria, with special reference to their fermentative reactions. J. Bact. **4**, 429–503.

WINSLOW, C.-E. A. AND ROGERS, ANNE F. 1905 A revision of the Coccaceae. Science, N. S. 21, pp. 669–672.

WINSLOW, C.-E. A. AND ROGERS, ANNE F. 1906 A statistical study of generic characters in the Coccaceae. J. Infect. Dis., **3**, 546.

WINSLOW, C.-E. A., ROTHBERG, WM. AND PARSONS, E. I. 1920 Notes on the classification of the white and orange staphylococci. J. Bact. **5**.

ZOPF, W. 1883–1885 Dis Spaltpilze. I–III Aufll. Breslau.

ZOPF, W. 1891 Über Ausscheidung von Fettfarbstoffen Lipochromen, seitens gewisser Spaltpilze. Ber. d. deutsch. bot. Gesellsch., **9**, 28.

THE PRODUCTION OF HYDROGEN SULPHIDE BY BACTERIA

JOHN T. MYERS

From the Department of Hygiene and Bacteriology, the University of Chicago

Received for publication, December 11, 1919

Hydrogen sulphide formation in sewage is supposed to be due to two distinct processes, the splitting of protein by certain organisms and the reduction of inorganic sulphates by others. Lederer (1913) concludes that more hydrogen sulphide is formed under anaerobic than under aerobic conditions. He thinks that the formation of hydrogen sulphide from protein is a selective process and that it depends a great deal on the position of the sulphur radical in the protein molecule. At any rate other factors than reduction are involved in hydrogen sulphide formation. An organism may be a very strong nitrate reducer and at the same time a weak hydrogen sulphide former, although both reactions are reducing processes. Lederer is of the opinion that the reduction of inorganic sulphates may however be an important factor in hydrogen sulphide formation, and thinks it probable that the action may be due to some unknown specific organism similar to the *Spirillum desulphuricans* of Beijerinck (1895).

Several workers have observed the formation of hydrogen sulphide in large quantities in the effluent of sewage disposal plants. Barr and Buchanan (1912) report the isolation of a specific organism in one such instance. Clark (1913) and others have reported the reduction of inorganic sulphates in sewages.

Sulphur metabolism may also play a beneficial rôle in sewage disposal in that certain species of bacteria may cause the oxidation of sulphur and of hydrogen sulphide to sulphates thus reducing the amount of odor. This is a well known property of such organisms as Beggiatoa and Thiothrix (Jordan, 1918).

Another field in which bacterial sulphur metabolism may be of importance is in soil bacteriology. The part played by sulphur here is little understood. Lipman, McLean and Lent (1916) suggest that sulphur oxidation in soils may have an effect on the availability of mineral phosphates as plant food.

Again a number of workers have attempted to apply hydrogen sulphide formation to water analysis, the assumption being that the amount of hydrogen sulphide produced when water is planted in a suitable medium is proportional to the degree of pollution. Schardinger (1894) seems to have first suggested this possibility. He observed that when water polluted with fecal material was added to peptone solution and incubated, it produced a characteristic odor, and that it blackened a strip of lead acetate paper which was suspended over the liquid. Dunham in 1897 suggested the following method for the detection of polluted water. Sterilize 10 cc. of an aqueous solution of 10 per cent peptone and 5 per cent sodium chloride in a plugged Erlenmeyer flask. To this flask add 90 cc. of the water under examination, suspend a strip of filter paper impregnated with lead carbonate over the mixture, and incubate for twenty-four hours at 37°C. Dunham maintained that the colon bacillus and the organisms of putrefaction readily multiply and cause the production of hydrogen sulphide which discolors the lead carbonate paper.

Redfield (1912) studied the effect upon the speed and amount of hydrogen sulphide production when different factors were varied. Among the conditions investigated were the effect of using filtered and unfiltered peptone solutions, the effect of the concentration of the peptone, of the type of inorganic salts added to the peptone solution, of the concentration of various salts added to the medium, of the kind of bases and acids used in adjusting the initial reaction of the medium, and of the relation of the final reaction of the culture to the amount of hydrogen sulphide production. As a result of his work Redfield suggests the following method for the detection of polluted water:

Bring 700 cc. of tap water to a boil and add 300 grams of Witte peptone and 75 grams of potassium chloride. Maintain a gentle heat and

stir constantly until as much of the peptone as will do so has gone into solution. Cool rapidly and make up to 1 liter with tap water. Transfer to a flask, heat to boiling again, plug, cool rapidly, and place in the ice box for at least twenty-four hours. Filter cold through paper and distribute in 10 cc. amounts among the special flasks suggested. Sterilize the flasks in the autoclave at one atmosphere for fifteen minutes. When cool, place in the tube of each flask a strip of bibulous paper 25 mm. in length, which had previously been impregnated with lead acetate.

The top of the plugged tube was then closed by wrapping with a strip of tin foil. The flasks alluded to were of a special design. They were shaped like a Sohxlet extraction flask, were graduated at 90 cc. and 100 cc. and a ground glass cap ending in a narrow tube was fitted over the neck of each flask.

Redfield tested this method by means of an artificial sewage, prepared from human feces. This sewage was quite dilute, since it contained only twenty colon bacilli per cubic centimeter and had a total bacterial count of twenty-eight hundred. He reports a gradual increase in the amount of hydrogen sulphide produced, and in the speed of its production as the concentration of sewage increases. The same result is reported with a considerable number of untreated waters from a variety of sources. He concludes that there is a uniform relationship between the degree of pollution of a water and the amount of lead acetate paper blackened when this method is employed.

Redfield also made some quantitative determinations of the amount of hydrogen sulphide produced by sewage organisms. He compared a number of methods for the quantitative determination of sulphur in peptone solutions. He also investigated the hydrogen sulphide producing powers of several species of bacteria, and concluded that the proteolytic organisms rather than *B. coli* are responsible for its formation.

Burnet and Weissenbach (1915) suggest another use for the hydrogen sulphide producing powers of bacteria. They found that colonies of *B. paratyphosus* B, became black when grown on agar which contained a small amount of lead acetate, while colonies of *B. paratyphosus* A did not. They consider this an accurate method for the differentiation of the two organisms.

Maymone (1917) suggests a somewhat similar method for the differentiation of *B. paratyphosus* A, and *B. paratyphosus* B based on biological characteristics and the appearance of cultures on lead acetate media.

Jordan and Victorson (1917) employed a like method for B. *paratyphosus* B, and *B. paratyphosus* A. Agar tubes containing a small amount of lead acetate were inoculated between the medium and the side of the tube. All typical strains of *B. paratyphosus* B blackened the needle track. The typical *B. paratyphosus* A strains produced no blackening. Kligler (1917) suggests a simple method for the differentiation of *B. paratyphosus* A and B, *B. typhosus*, and *B. dysenteriae*, based on a double sugar medium similar to that of Russell, containing lead acetate.

Bacterial action probably plays a rôle in the formation of intestinal gases. Hydrogen sulphide is always present in intestines and is probably formed from cystin. Senator described a case in which an intoxication with hydrogen sulphide of intestinal origin occurred, but this is apparently the only case reported. (Wells 1918). Hydrogen sulphide formation by bacteria may be concerned in some of the conditions included in that vague term, autointoxication.

The formation of hydrogen sulphide by bacteria is also of interest, because of the light it may throw on the metabolism of bacteria. Sasati and Otsuka (1912) carried out some experiments with a few organisms as to the formation of hydrogen sulphide with cystin, taurin, sulphur, sodium, sulphate, and sodium sulphite. Burger (1914) compared cystin and peptone as a source of hydrogen sulphide.

Tanner (1918) published interesting data on various sulphur compounds as a source of hydrogen sulphide when acted on by bacteria. He (1918) has also contributed a valuable article on the formation of hydrogen sulphide by yeasts.

EXPERIMENTAL WORK

The application of hydrogen sulphide formation to water analysis

Experiments were undertaken to ascertain the delicacy and reliability of the hydrogen sulphide test used by Redfield to determine the potability of water.

The medium used was that suggested by Redfield with minor modifications. Sodium chloride was substituted for potassium chloride, and instead of the special flasks which Redfield employed, Erlenmeyer flasks of 150 cc. capacity and made of Pyrex glass were used. One-half per cent sodium chloride was added to all media containing peptone because it seemed to produce a clearer medium. Difco peptone made by the Digestive Ferments Company was substituted for Witte peptone since the latter is not now obtainable. Ten cubic centimeters of the medium were placed in each flask and autoclaved for ten minutes at 15 pounds pressure. A strip of filter paper 50 mm. long and 3 mm in width was suspended in the mouth of the flask in such a manner that approximately 30 mm. of the strip was exposed. The filter paper had been moistened in a 10 per cent solution of neutral lead acetate, and sterilized in the autoclave for fifteen minutes at 15 pounds pressure. It was necessary to sterilize the lead acetate paper separately because it was found that some blackening occurred when the medium was autoclaved, due probably to a slight hydrolysis of the peptone, since steam at the pressure of the autoclave ionizes more than at atmospheric pressure.

One gram of the feces under examination was weighed out. This merely served as a convenient amount from which to make dilutions since of course the moisture, bacterial content and residue varied enormously in the different samples. A 90 cc. portion of each dilution studied was placed in one of the Erlenmeyer flasks and incubated aerobically at 37°C. The approximate number of millimeters of lead acetate paper blackened was recorded at the end of twenty-four hours, forty-eight hours, seventy-two hours, and seven days. The method was of course, only approximate, but all conditions were kept as nearly identical as

possible. The total forty-eight hour 20°C. bacterial count on standard agar, the total forty-eight hour 37°C. bacterial count on standard agar, and the colon count, using the complete test fermentation tube method, were made in each case (A. P. H. A. Committee, 1912, 1917). In the fermentation tube work, ten tubes of lactose broth were inoculated with 1 cc. each of the dilution selected and several dilutions were always used in order to make sure that some one series of ten tubes would show gas in only part of the tubes. All media were prepared according to the standard method of water analysis of the American Public Health Association (1912, 1917).

The colon count was also made in each sample by plating on Endo medium and counting the colon like colonies directly. This was done as a check on the fermentation tube method and to test the possibility of making direct counts of the colon content of sewage and other materials by the use of Endo medium.

Table 1 indicates the types of feces studied and the data obtained.

The counts are expressed in millions and fractions of millions in order to make the table less bulky. Table 1 indicates that there is a fairly definite relationship between the amount of fecal material in a given solution and the amount of hydrogen sulphide formed, although occasional irregularities appear.

There were some difficulties in making a colon count directly on Endo medium, the most important being that deep colonies are at times difficult to differentiate. On the whole it would seem that the method is as accurate as the fermentation tube method and it is perhaps less cumbersome, especially when dealing with material that is heavily loaded with colon bacilli.

Table 2 gives the minimum number of colon bacilli which produced a perceptible blackening of the lead acetate paper after incubation at 37°C. for twenty-four hours, forty-eight hours, and seven days respectively. It also gives the twenty-four hour 37°C. count, and the forty-eight hour 20°C. count for purposes of comparison with the colon count. The customary 90 cc. dilution was used.

There is no constant relationship between the number of colon bacilli from different animals and the amount or rate of hydrogen sulphide production; and there is also no definite relationship between the numbers of other organisms and hydrogen sulphide

TABLE 1

Summary of examination of feces from different animals

SOURCE	37°, 24-hour STANDARD AGAR COUNT IN MILLIONS PER GRAM	20°, 48-HOUR STANDARD AGAR COUNT IN MILLIONS PER GRAM	COLON COUNT IN MILLIONS PER GRAM ON ENDO MEDIUM	COLON COUNT IN MILLIONS PER GRAM BY FERMENTATION TUBE	APPROXIMATE NUMBER OF MILLIMETERS OF LEAD ACETATE PAPER BLACKENED											
					Dilution = 1:100000			Dilution = 1:1000000			Dilution = 1:10000000			Dilution = 1:100000000		
					24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Human	39.8	20.9	33.0	30.0	2	30	30	1	5	30	0	0	0	0	0	0
	27.5	6.1	8.0	11.0	0.5	7	30	0	7	30	1	2	4	0	0	0
Bovine	1.74	1.25	0.01	0.1	1	3	5	0.5	1	1	0	0	0	0	0	0
	2.5	1.8	7.5	5.0	4	10	30	1	5	30	0	1	6	0	0	0
Horse	0.31	0.25	0.07	0.1	7	30	30	3	10	25	0	1	2	0	0	0
	55.0	1.0	0.06	0.08	1	30	30	0	12	30	0	1	4	0	0	0
Sheep	90.0	11.0	10.0	5.0	10	15	30	2	7	10	2	4	30	0	1	4
	224.0	215.0	210.0	90.0	3	3	20	1	2	6	0	0	1	0	0	0
Pig	288.0	281.0	233.0	90.0	—	—	—	10	30	20	4	10	30	0.5	1	2
	90.0	50.0	10.0	61.0	2	30	30	1	30	30	9	2	2	0	0	0
Chicken	126.0	26,000.0	20.0	10.0	—	—	—	10	30	30	10	15	30	1	2	5
	28.0	32.0	2.9	4.0	10	30	30	3	20	30	0	15	30	0	0	10
Rabbit*	3.2	2.23	0.01	0.003	1	4	30	1	5	5	0	0	0	0	0	0
	17.0	20.9	0.04	0.01	1	20	30	0	1	30	0	15	30	0	10	30
Dog	53.0	48.0	47.0	50.0	10	15	20	10	15	30	5	20	30	0	20	30
	35.0	34.0	11.0	6.0	—	—	—	5	20	30	5	20	30	0	20	30

* The dilution is one-tenth lower than that given in the column heading.

formation. Certain types of animal feces seem to produce hydrogen sulphide in even higher dilutions than human feces. Therefore this test applied to the direct examination of water would have no value since it is too delicate. It is to be expected

TABLE 2

Relation between the number of bacteria, in the 90 cc. portion of the highest dilution of various types of feces, which produced hydrogen sulphide

SOURCE	COLON BACILLI			37°, 24-HOUR COUNT ON STANDARD AGAR			20°, 48-HOUR COUNT ON STANDARD AGAR		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Human	2,700	2,700	2,700	3,580	3,580	3,580	1,880	1,880	1,880
	99	99	10	22,750	2,275	227	5,490	549	55
Bovine	9	9	9	157	157	157	110	110	110
	450	45	45	225	22	22	162	16	16
Horse	1	0	0	28	3	3	23	2	2
	1	0	0	4,950	4,950	4,950	90	90	90
Sheep	45	5	1	810	81	1	100	10	1
	8,100	8,100	810	20,160	20,160	2,016	19,350	19,350	1,935
Pig	5,490	549	549	8,100	810	810	4,500	450	450
	210	2	2	160	2	2	153	2	2
Chicken	2	0	0	113	1	1	23,000	230	230
	360	36	4	2,520	252	25	280	28	3
Rabbit	30	3	3	28,800	2,880	2,880	20,000	2,000	2,000
	90	90	90	158,000	158,000	158,000	18,800	18,800	18,800
Dog	4	4	4	5	5	5	4	4	4
	54	—	—	315	—	—	306	—	—

that results of this type would be irregular since many other bacteria aside from colon bacilli are capable of producing hydrogen sulphide. It would be interesting to determine whether there is any difference in the hydrogen sulphide forming power of pure cultures of colon bacilli coming from different sources.

Table 3 summarizes the results of a rather complete bacteriological analysis of samples of water from various sources, the purpose being to compare the hydrogen sulphide test with standard methods.

The samples marked "University of Chicago tap" were specimens from the Chicago mains. Those marked "University of Chicago filtered" were taken from the University of Chicago drinking fountains, the water having been passed through a special filter belonging to the University. It will be noted that the condition of these waters was very good according to the usual standards, yet a small amount of hydrogen sulphide appeared in one of the filtered samples.

The samples marked Omaha House 1, 2, 3, 4, 5, and 6, were taken from the taps of houses in a certain district of Omaha, where several cases of typhoid fever had occurred. These analyses were made to determine the condition of the city water supply. Two series of examinations were made about two weeks apart. There was no evidence of contamination according to standard methods yet hydrogen sulphide was formed in two instances in twenty-four hours, and in most other samples there was an appreciable amount of blackening in forty-eight hours.

These results would indicate that this test is too delicate to be of value in water analysis. Hydrogen sulphide was formed by every contaminated water and by some waters in which there was no evidence of contamination by the usual methods of examination. This agrees with the results obtained by examination of feces from various animals.

TABLE 3

Summary of water analyses

SOURCE	37°, 24-HOUR STANDARD AGAR COUNT PER CUBIC CENTIMETER	20°, 48-HOUR STANDARD AGAR COUNT PER CUBIC CENTIMETER	COLON COUNT PER CUBIC CENTIMETER BY FERMENTATION TUBE METHOD	COLON COUNT ON ENDO'S MEDIUM	APPROXIMATE NUMBER OF MILLIMETERS OF LEAD ACETATE PAPER BLACKENED								
					Dilution = 0			Dilution = 1:10			Dilution = 1:100		
					24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
University of Chicago tap	150	200	0	0	0	0	0	—	—	—	—	—	—
University of Chicago tap. filtered	70	170	0	0	0	0	1	—	—	—	—	—	—
Hobart, Ind.	100	220	0	0	0	0	2	—	—	—	—	—	—
Gary, Ind., Depot	46	75	0.3	0	0	0	0	—	—	—	—	—	—
Gary, Ind., Hotel, No. 1	41	70	0	0	0	0	0	—	—	—	—	—	—
Gary, Ind., Hotel, No. 2	60	90	0	0	0	0	0	—	—	—	—	—	—
Gary Depot	30	60	0	0.1	2	4	30	—	—	—	—	—	—
Gary Hotel No. 1	60	40	0	0	2	5	30	—	—	—	—	—	—
Gary Hotel No. 2	45	65	0	0	0	0	0	—	—	—	—	—	—
Gary Depot	60	100	0	0	0	1	3	—	—	—	—	—	—
Gary Hotel No. 1	55	85	0.2	0.4	1	2	3	—	—	—	—	—	—
University of Chicago tap	20	150	0.2	0.4	1	2	3	—	—	—	—	—	—
Lake Geneva, Wis.	32	2,600	0	0	0	0	0	0	0	0	0	0	—0
University of Chicago tap	32	48	0.1	0	0	0	0	—	—	—	—	—	—
University of Chicago tap, filtered	4	3	0.1	0	1	10	10	—	—	—	—	—	—
University of Chicago tap	65	410	0	0.3	30	30	30	—	—	—	—	—	—
University of Chicago tap, filtered	2	16	0	0	0	0	0	—	—	—	—	—	—
University of Chicago tap	41	140	0	0	0	0	0	—	—	—	—	—	—
University of Chicago tap, filtered	2	15	0	0	0	0	0	—	—	—	—	—	—
University of Chicago tap	25	400	0	0	0	0	0	—	—	—	—	—	—
University of Chicago tap, filtered	2	38	0	0	0	0	0	—	—	—	—	—	—

Omaha house No. 1 tap	12	7	0	0	0	0	6	0	0	0.5	–	–	–
Omaha house No. 2 tap	14	11	0	0	0	30	30	0	0	30	–	–	–
Omaha house No. 3 tap	2	6	0	0	1	6	30	0	0.5	4	–	–	–
Omaha house No. 4 tap	34	10	0	0	0	12	30	0	0	0	–	–	–
Omaha house No. 5 tap	10	4	0	0	0	4	30	0	0	0	–	–	–
Omaha house No. 6 tap	19	10	0	0	0	6	30	0	0	0	–	–	–
University of Nebraska tap	125	50	0	0	1	2	5	0.5	1	2	0	0.5	–
Omaha house No. 1 tap	9	14	0	0	0	0	0	0	0	0	–	–	–
Omaha house No. 2 tap	9	11	0	0	0	0	0	0	0	0	–	–	–
Omaha house No. 3 tap	7	10	0	0	0	6	30	0	4	10	–	–	–
Omaha house No. 4 tap	12	10	0	0	0	0	0	0	0	0	–	–	–
Omaha house No. 5 tap	13	14	0	0	0	4	10	0	0	0	–	–	–
University of Nebraska tap	8	20	0	0	0	5	30	0	0	0	–	–	–
Well water	870	900	0	0	0	0	0	0	0	0	–	–	–
Calumet River	4,400	–	90	110	–	–	–	1	2	30	1	2	30
Men's Natatorium, University of Chicago	76,000	103,000	60	17	1	12	30	0.5	5	5	0	1	1
Women's Natatorium, University of Chicago	420,000	100,000	10	10	30	30	30	2	4	6	3	4	4
Men's Natatorium, University of Chicago	650	20,000	4	2	4	30	30	0.5	2	2	0	0	0

Effect of the variety of commercial peptone, and of the presence of glucose and sucrose on hydrogen sulphide formation

The hydrogen sulphide producing power of a number of common bacteria was studied in several media. Witte's peptone, "Difco" peptone made by the Digestive Ferments Company, and Fairchild peptone were compared. Four different media were prepared from each brand of peptone, a 3 per cent solution of the peptone containing 0.5 per cent of NaCl, a 3 per cent solution of the peptone containing 0.5 per cent NaCl and 0.5 per cent glucose, a 3 per cent solution of the peptone containing 0.5 per cent NaCl and 0.5 per cent sucrose, and standard nutrient broth. Glucose and sucrose were chosen because Seifert (1909) reports that glucose decreases and sucrose increases hydrogen sulphide formation by B. *paratyphosus B.* Table 4 summarizes the results.

Witte's peptone was the most favorable of the three brands in regard to hydrogen sulphide formation, and Fairchild's was more favorable than Difco. Variable amounts of hydrogen sulphide were formed in Witte and not in Difco peptone media by *Sarcina lutea, Staphylococcus albus, Staphylococcus aureus, B. prodigiosus, B. pyocyaneus, Mic. tetragenus,* a laboratory strain of streptococcus, *B. cloacae, B. subtilis, B. anthracis, B. avisepticus, B. bovisepticus, Sp. metchnikovii, Sp. cholerae, B. lactis-aerogenes,* and *B. mucosus-capsulatus.* Larger amounts of hydrogen sulphide were formed in Witte than in Difco peptone by some of the other organisms.

Hydrogen sulphide was produced in Fairchild and not in Difco media by *B. lactis aerogenes* and *B. cloacae.* It seemed to make no difference which of the four media prepared from each peptone was used. Ordinary broth was as good as any. Glucose and sucrose did not influence the rate or amount of hydrogen sulphide formation.

It was a striking fact that many species of bacteria produced hydrogen sulphide from Witte's peptone and not from the American brands. This was of course due to difference in chemical constituents. Witte's peptone contains more amino

TABLE 4

Relative amount of hydrogen sulphide produced by various bacteria in Witte peptone media

ORGANISM	3% PEPTONE 0.5% NaCl			3% PEPTONE 0.5% NaCl 0.5% GLUCOSE			3% PEPTONE 0.5% NaCl 0.5% SUCROSE			BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Sarcina lutea	0	0	1	0	0	0	0	0	2	0	1	1
Staph. aureus	0	0	1	0	7	30	0	7	30	1	4	22
Staph. albus	2	5	12	3	5	10	3	5	8	8	10	20
B. prodigiosus	2	3	12	2	4	7	14	22	30	1	2	12
B. pyocyaneus	0	0	1	0	0	0	0	0	1	0	1	2
Mic. tetragenus	0	0	0	0	0	0	0	0	0	0	1	3
Strep. pyogenes	2	2	2	25	27	27	17	25	30	2	2	2
B. coli	5	15	30	19	20	20	19	22	30	15	25	28
B. lactis aerogenes	2	2	10	19	25	27	15	25	30	3	6	20
B. mucosus capsulatus	0	2	5	6	12	15	4	20	30	3	3	5
B. enteritidis	9	15	27	13	15	20	8	17	30	12	30	30
B. para-typhosus B	9	18	22	7	11	15	7	9	15	15	20	25
B. para-typhosus A	0	0	1	0	0	1	0	0	2	0	0	3
B. typhosus	5	9	15	4	12	15	5	15	25	15	25	30
B. dysenteriae Shiga	0	0	0	0	0	0	0	0	1	0	1	2
B. dysenteriae Flexner	0	0	1	1	1	3	0	0	1	0	1	3
B. proteus-vulgarus	22	27	30	22	25	30	18	27	28	30	30	30
B. cloacae	5	20	27	15	25	30	17	30	30	4	11	25
B. subtilis	30	30	30	22	27	30	22	27	30	25	27	27
B. anthracis	30	30	30	25	30	30	25	30	30	30	30	30
B. moelleri	0	0	0	0	0	2	0	1	2	0	1	3
B. fecalis-alkaligenes	0	1	2	0	1	2	0	1	3	4	6	14
B. bovisepticus	3	3	3	16	18	30	19	25	30	3	3	3
B. avisepticus	2	4	6	12	22	25	0	1	3	9	18	23
Sp. metchnikovii	0	12	25	0	0	0	0	1	25	8	22	27
Sp. cholerae	6	25	28	7	8	9	19	22	30	13	27	30
B. hofmanni	0	0	0	0	0	0	0	0	0	0	0	1

Relative amount of hydrogen sulphide produced by various bacteria in "Difco" peptone media

Sarcina lutea	0	0	0	0	0	0	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0	0	0	0	0	0	0
Staph. albus	0	0	0	0	0	0	0	0	0	0	0	1
B. prodigiosus	0	0	0	0	0	0	0	0	2	0	0	0
B. pyocyaneus	0	0	0	0	0	0	0	0	0	0	0	0
Mic. tetragenus	0	0	0	0	0	0	0	0	0	0	0	0
Strep. pyogenes	0	0	0	0	0	0	0	0	0	0	0	0
B. coli	0	1	1	1	1	2	1	1	2	1	1	1
B. lactis aerogenes	0	0	0	0	0	0	0	0	0	0	0	0
B. mucosus capsulatus	0	0	0	0	0	0	0	0	0	1	1	1
B. enteritidis	15	30	30	4	20	20	4	4	4	22	30	30
B. para typhosus B	10	30	20	12	25	25	6	15	15	22	27	27
B. para typhosus A	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 4—*Continued*

ORGANISM	3% PEPTONE 0.5% NaCl			3% PEPTONE 0.5% NaCl 0.5% GLUCOSE			3% PEPTONE 0.5% NaCl 0.5% SUCROSE			BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
B. typhosus	25	30	30	0	0	0	4	6	7	25	30	30
B. dysenteriae Shiga	0	0	0	0	0	0	0	0	0	0	0	0
B. dysenteriae Flexner	0	0	0	0	0	0	0	0	0	0	0	0
B. proteus vulgarus	5	10	20	7	10	10	5	6	6	25	30	30
B. cloacae	0	0	0	0	0	0	0	0	1	0	0	1
B. subtilis	0	0	0	0	0	0	0	0	0	0	0	0
B. anthracis	0	0	0	0	0	0	0	0	0	1	1	1
B. moelleri	0	0	5	0	0	0	0	0	0	0	0	0
B. fecalis alkaligenes	0	0	0	0	0	0	0	0	1	0	0	1
B. bovisepticus	0	0	0	0	0	0	0	0	1	0	0	0
B. aviseptieus	0	0	0	0	0	0	0	0	0	20	25	25
Sp. metchnikovii	0	0	0	0	0	0	0	0	1	0	0	0
Sp. cholerae	0	0	0	0	0	0	0	0	0	0	0	0
B. hofmanni	0	0	0	0	0	0	0	0	0	0	0	0
Relative amount of hydrogen sulphide produced by various bacteria in Fairchild peptone media												
Sarcina lutea	0	1	5	0	0	2	0	0	0	0	0	0
Staph. aureus	0	1	1	0	0	1	0	0	0	0	0	1
Staph. albus	0	0	0	0	0	1	0	0	0	1	1	4
B. prodigiosus	0	0	2	0	0	1	0	0	1	0	1	2
B. pyocyaneus	0	0	0	0	0	0	0	0	0	0	0	0
Mic. tetragenus	0	0	0	0	0	0	0	0	0	0	0	0
Strep. pyogenes	0	0	0	0	0	0	0	0	0	0	0	0
B. coli	2	2	3	1	1	2	0	0	1	1	1	2
B. lactis-aerogenes	1	2	3	0	1	2	0	0	1	0	1	6
B. mucosus-capsulatus	0	0	0	0	0	0	0	0	1	0	0	0
B. enteritidis	22	30	30	1	2	3	11	20	27	3	20	30
B. para-typhosus B	25	30	30	26	30	30	15	20	27	15	18	27
B. para-typhosus A	0	0	0	0	0	0	0	0	0	0	0	0
B. typhosus	15	22	25	1	1	2	15	20	27	1	1	15
B. dysenteriae Shiga	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae Flexner	0	0	1	0	0	0	0	0	0	0	0	0
B. proteus vulgarus	25	27	30	25	27	27	11	17	27	1	5	15
B. cloacae	1	2	5	0	1	4	0	0	1	1	2	2
B. subtilis	0	0	0	0	0	0	0	0	0	0	0	0
B. anthracis	0	0	0	0	0	0	0	0	0	0	0	0
B. moelleri	0	1	1	1	1	3	0	0	5	0	0	0
B. fecalis-alkaligenes	0	0	0	0	0	1	0	0	0	0	1	1
B. bovisepticus	2	2	2	0	0	0	0	0	0	0	0	0
B. aviseptieus	0	5	5	0	0	0	0	0	0	0	0	0
Sp. metchnikovii	0	0	0	0	0	0	0	0	0	0	0	0
Sp. cholerae	0	0	0	0	0	0	0	0	0	0	0	0

acids than the American products. It probably also has a higher percentage of sulphur bearing constituents, possibly cystin. It would be interesting to determine the relative amounts of cystin in each type of peptone, but this has not yet been done.

A series of experiments was carried out to study the effect of various carbohydrates on hydrogen sulphide formation, by *B. paratyphosus* B. A medium made up of 3 per cent. Witte peptone, 0.5 per cent NaCl and 0.5 per cent of the carbohydrate was used. It was sterilized for twenty minutes in the Arnold on three consecutive days. Three monosaccharides, glucose, levulose and galactose; two disaccharides, lactose and sucrose; and a glucoside salicin were tried. No marked constant effect of these different carbohydrates on hydrogen sulphide formation was noted; this is not quite in accord with the observations of Seifert (1909) who found that the presence of glucose and lactose in peptone media decreased the amount of hydrogen sulphide formed.

Hydrogen sulphide production by B. Paratyphosus A and B. Paratyphosus B. and B. typhosus and B. dysenteriae

A number of strains of *B. paratyphosus* A, *B. paratyphosus* B. *B. typhosus*, and *B. dysenteriae*, were procured. Some of the cultures were obtained from the University of Chicago, some from the University of Kansas, some from the American Museum of Natural History, New York City, and some were freshly isolated from blood or stools at the University of Nebraska, College of Medicine. Their power of producing hydrogen sulphide was tested in a 3 per cent solution of Witte's peptone containing 0.5 per cent of NaCl, the same medium made from Difco and from Fairchild's peptones, respectively, and broth prepared from Difco peptone. All media were sterilized in the Arnold. The results are recorded in tables 5 and 6.

The tables need but little comment. So far as the cultures investigated were concerned *B. typhosus* always produced large amounts of hydrogen sulphide in twenty-four hours or less, while

B. dysenteriae never produced appreciable quantities in twenty-four hours and only a few strains produced traces after a week's incubation. The same distinction held for *B. paratyphosus* A and B. *paratyphosus B.* B always forming hydrogen sulphide in twenty-four hours or less and A forming practically none even

TABLE 5

Relative amount of hydrogen sulphide production by various strains of B. paratyphosus A, and B. paratyphosus B

ORGANISM	1% WITTE PEPTONE 0.5% NaCl			1% FAIRCHILD PEPTONE 0.5% NaCl			1% DIFCO PEPTONE 0.5 NaCl			DIFCO BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
B. paratyphosus B. 8	15	20	30	22	27	30	30	30	30	25	25	30
B. paratyphosus B. 12	7	10	30	22	25	30	27	30	30	22	30	30
B. paratyphosus B. 179	7	7	12	5	8	10	3	5	8	10	12	12
B. paratyphosus B. 101	12	20	25	25	27	30	30	30	30	20	22	25
B. paratyphosus B. 180				22	27	30	30	30	30	25	25	28
B. paratyphosus B. 323	10	20	30	22	30	30	30	30	30	27	30	30
B. paratyphosus B. 22	8	10	15	20	25	25	30	30	30	30	30	30
B. paratyphosus B. 138	9	17	25	15	22	30	30	30	30	25	30	30
B. paratyphosus A. 3	0	1	2	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 158	0	1	1	0	0	0	0	0	1	0	0	0
B. paratyphosus A. 9	0	1	1	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 294	0	0	0	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 16				0	0	0	0	0	0	0	0	1
B. paratyphosus A. 322	0	0	0	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 295	0	0	1	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 10	0	0	0	0	0	0	0	0	0	0	0	0
B. paratyphosus A. 4	0	0	0	0	0	1	0	0	0	0	0	0

after a week's incubation. This property, combined with the use of Endo and Russell's medium, should be of considerable value in differentiating these organisms, especially when the detection of carriers is the object in view. Ordinary broth is as satisfactory for this purpose as any medium tried. Sterilization in the autoclave instead of in the Arnold is satisfactory.

TABLE 6

Relative amount of hydrogen sulphide production by different strains of typhoid and dysentery bacilli

ORGANISM	1% WITTE PEPTONE 0.5% NaCl			1% FAIRCHILD PEPTONE 0.5% NaCl			1% DIFCO PEPTONE 0.5% NaCl			DIFCO BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Typhosus, Hopkins	8	18	25	15	20	30	27	27	30	15	20	20
Typhosus, No. 190	10	20	25	12	22	23	30	30	30	22	22	22
Typhosus, Omaha No. 1	7	15	20	8	12	15	25	25	25	25	25	25
Typhosus, Omaha No. 2	12	16	23	10	15	20	20	25	30	20	25	25
Typhosus, Omaha No. 3	9	13	20	7	15	15	25	27	27	22	25	27
Typhosus, Omaha No. 4	10	16	22	9	15	20	27	27	27	18	25	30
Typhosus, Omaha No. 5	12	20	25	10	15	20	25	30	30	15	25	27
Typhosus, Omaha No. 6	10	18	23	10	15	22	22	25	25	20	25	25
Typhosus, No. 189	9	17	25	12	18	18	27	27	30	20	22	25
Typhosus, No. 197	9	20	22	14	17	22	30	30	30	17	20	27
Typhosus, No. 607				5	15	15	20	25	25	20	25	25
Typhosus, No. 11	10	15	20	9	11	15	30	30	30	15	20	25
Typhosus, No. 608				7	12	12	30	30	30	18	20	20
Typhosus, 5	9	20	25	10	15	15	30	30	30	18	20	22
Typhosus, Cary	12	18	25	15	18	20	20	25	25	20	22	25
B. dysenteriae, Flexner C	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, Hofmann C	0	0	2	0	0	0	0	0	0	0	0	1
B. dysenteriae Shiga. W	0	0	0	0	0	0	0	0	0	0	0	0
B. dysenteriae Shiga. C	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae Strong	0	0	0	0	0	0	0	0	0	0	0	0
B. dysenteriae, Flexner W	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, 78 W	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, Omaha No. 1	0	0	1	0	0	0	0	0	0	0	0	0

The relationship between chemical structure and hydrogen sulphide formation

In order to study the relationship between the state of oxidation of sulphur compounds and the ease with which they may be attacked by bacteria with resulting hydrogen sulphide formation, three compounds were selected. Sodium sulphate was chosen as a representative inorganic sulphur compound in which

the sulphur is fully oxidized. Taurin was taken as a representative of the sulphur components of proteins in which the sulphur is fully oxidized. This compound is amino ethyl sulphonic acid $\begin{matrix} CH_2\,NH_2 \\ | \\ CH_2\,SO_3H, \end{matrix}$ analogous to a sulphate except that one oxygen is replaced by an H. It is also an amino acid. Cystin the disulphide of diamino thiolactic acid was selected because it is an amino acid.

$$\begin{matrix} CH_2S - S - CH_2 \\ | \qquad\quad | \\ | \qquad\quad | \\ CH \cdot NH_2 \quad CHNH_2 \\ | \qquad\quad \| \\ COOH \qquad COOH \end{matrix}$$

Here the sulphur is in the reduced state. Cystin is the usual sulphur-containing amino acid found in proteins. Cystin and taurin are analogous except as to the state of oxidation of the sulphur.

A medium was prepared as follows: ammonium tartrate 10 grams, anhydrous sodium sulphate, 10 grams, and distilled water to 1000 cc. The mixture was put in culture tubes and autoclaved for ten minutes at 15 pounds pressure. Five organisms were used, *B. coli* and *B. paratyphosus* B, because they are hydrogen sulphide formers which have no proteolytic action when tested in the ordinary media, and *B. fluorescens-liquifaciens*, *B. cloacae*, and *B. proteus-vulgaris*, because they are organisms, with varying degrees of proteolytic activity. Duplicate cultures were made, strips of sterile lead acetate paper were inserted in the mouth of the tubes, and they were placed in a moist 37° incubator. No growth appeared in any of the tubes at the end of seven days incubation under aerobic conditions.

An exactly similar medium to the one given above was made, except that 10 grams of chemically pure glucose was added. After seventy-two hours incubation a vigorous growth appeared in all of the tubes but even after fourteen days incubation there was no perceptible blackening of the lead acetate paper. Evi-

dently carbohydrate was a necessary source of carbon for the metabolism of the bacteria. It was omitted in the first medium in order to learn whether the carbon in the ammonium tartrate could serve this purpose.

Taurin was prepared by the method of Hawk (1918). Its purity was determined by making duplicate analyses for sulphur using the method of Redfield (1912). Theory called for 26.15 per cent and we found 25.90 per cent.

A 0.5 per cent solution of taurin was made in distilled water. It was sterilized with a Berkefeld filter because sterilization by means of heat caused blackening of lead acetate paper, and pointed toward a chemical change in the taurin. Filtration obviated any such possibility.

Duplicate tubes were inoculated with the same series of organisms which were used with the sodium sulphate medium; these were incubated for one week under areobic conditions at 37°C. without growth.

Another medium was prepared, identical with the above except that 1 per cent of chemically pure glucose was added. After one week's incubation a slight growth appeared in the tubes inoculated with *B. proteus-vulgaris*, and *B. fluorescens-liquifaciens*, but not in the others. No blackening of the paper occured in any instance. This would indicate that taurin is not readily attacked by bacteria.

Cystin was prepared by the method of Matthews and Walker (1909).

Its purity was ascertained by determining the percentage of sulphur by the same method used for the taurin. Theory called for 26.72 per cent; we found, 27.04 per cent.

An attempt was made to prepare the ammonium salt of cystin by dissolving the cystin in ammonium hydroxide and evaporating the excess ammonia. The cystin precipitated as soon as the fumes of the ammonia disappeared. Evidently the ammonium salt was easily hydrolyzed.

The citrate of cystin acted in the same way. In order to keep it in solution an excess of citric acid was required which was sufficient to interfere with bacterial growth.

Cystin plus tartaric acid behaved in a similar manner.

Finally a 0.5 per cent. solution of cystin was prepared by adding just enough sodium carbonate to keep the cystin in solution. The medium was sterilized by filtration because heat caused hydrogen sulphide formation. It was placed in sterile tubes and tested for sterility. Tubes were inoculated with the series of organisms used with the sodium sulphate and taurin mediums. After a week's incubation no growth appeared.

Another series of tubes were inoculated with the same bacteria used previously. A bit of sterile litmus paper was added to each tube and sterile 5 per cent. hydrochloric acid added till the litmus was faintly red. The cystin was precipitated as the neutral point was approached but this was ignored. After three days incubation the litmus turned blue and sterile acid was again added till it turned faintly red. Growth appeared in all the tubes and hydrogen sulphide was formed in some of them. Table 7 gives the results.

TABLE 7

Formation of hydrogen sulphide from a solution of cystin, in millimeters of lead acetate paper blackened

ORGANISM	24 HOURS	48 HOURS	72 HOURS	7 DAYS
B. paratyphosus B	0	2	7	7
B. typhosus	0	0	0	0
B. cloacae	0	0	0	0
B. proteus	1	2	4	25
B. fluorescens liquifaciens	0	0	15	25

This experiment was repeated except that the cystin was dissolved in the smallest possible amount of tenth normal hydrochloric acid and tenth normal sodium carbonate was added till a precipitate formed and the medium was only slightly acid, the exact reverse of the preceding method. The results were the same.

Cystin could doubtless have been sterilized by dissolving it in an organic solvent, evaporating this off and adding sterile distilled water but this was not tried.

Probably nearly neutral solutions of cystin in citric acid, in tartaric acid, or in ammonium hydroxide, prepared as in the case of the sodium carbonate solution, would have given similar results but these methods were not tried. The work done seemed to indicate that organic sulphur in the partially reduced condition rather than in the oxidized forms gives rise to hydrogen sulphide when ordinary bacteria act on protein.

SUMMARY

The examination of a number of samples of feces showed no constant difference between the amount of hydrogen sulphide produced by human and animal fecal material. This indicates that a test of samples of water for hydrogen sulphide production would be of no value in distinguishing between human and animal contamination.

The hydrogen sulphide test is too delicate for use in examination of water for the detection of fecal contamination of any type. All contaminated waters examined were positive to this test and many were positive which gave no evidence of contamination by the usual criteria.

A considerable number of common bacteria were able to produce hydrogen sulphide from Witte peptone and not from Difco peptone and some other kinds were able to produce it in larger amounts. Fairchild's peptone yielded more hydrogen sulphide than did Difco peptone. This emphasizes the need for uniform media.

Glucose and lactose had little effect on hydrogen sulphide formation. There was little difference between 3 per cent. peptone solutions and standard beef extract broth.

All strains of *B. paratyphosus* B examined, produced hydrogen sulphide in twenty-four hours or less, while none of the strains of *B. paratyphosus* A were able to do this.

All strains of *B. typhosus* studied produced hydrogen sulphide in twenty-four hours or less and none of the strains of *B. dysenteriae* had this power.

Sodium sulphate was not a source of hydrogen sulphide in the limited number of experiments made.

Taurin was attacked only to a very limited extent by the bacteria used, and none of them were able to split it to the extent of formation of hydrogen sulphide.

Cystin in distilled water was able to support bacterial growth in the case of some of the common bacteria, and even in some instances to permit of hydrogen sulphide formation. This indicates that oxidized sulphur is not readily attacked by bacteria while partially reduced sulphur is completely reduced to hydrogen sulphide.

REFERENCES

BARR AND BUCHANAN 1912 Iowa State College, Eng. Exper. Sta. Bull. 26.

BEIJERINCK 1895 Centralbl. f. Bakt. 2 Abt., **1**, 3.

BURGER 1914 Arch. f. Hyg. **82**, 201–11.

BURNET AND WEISSENBACH 1915 Compt. rend. de Soc. de Biol., **78**, 565–568.

CLARK 1913 Rept. Mass. State Bd. of Health, 251.

DUNHAM 1897 J. Am. Chem. Soc., **19**, 591.

HAWK 1918 Practical Physiological Chemistry. 6th ed., 214.

HEINEMANN 1915 A Laboratory Guide in Bacteriology, 3rd ed., 86.

JORDAN, E. O. 1918 General Bacteriology, 6th ed., 105.

JORDAN, E. O., AND VICTORSON, RUTH 1917 J. Infect. Dis., **21**, 554–5.

KLIGLER, I. J. 1917 Am. J. Pub. Health, **7**, 805.

LEDERER 1913 Am. J. Pub. Health, **3**, 552–61.

LIPMAN, MCLEAN AND LENT 1916 Soil Science, **2**, 499–538.

MATTHEWS AND WALKER 1909 J. Biol. Chem., **6**, 21–37.

MAYMONE 1917 Arch. Sci. Med., **4**, 202–234.

REDFIELD 1912 Hydrogen sulphide production and its significance in the sanitary examination of water. Thesis for Ph.D. Degree, Cornell.

SATI AND OTSUKA 1912 Biochem. Ztschr., **39**, 208–215.

SCHARDINGER 1894 Centralbl. f. Bakt., **16**, 853–859.

SEIFERT 1909 Zeit. f. Hyg., **63**, 273–290.

Standard Methods of Water Analysis. 1912 and 1917. Am. Pub Health Assoc.

TANNER, F. W. 1918a J. Bact., **2**, 585–593.

TANNER, F. W. 1918b J. Amer. Chem. Soc., **40**, 663–669.

WELLS 1918 Chemical Pathology, 3d ed., 581.

A CORRELATION STUDY OF THE COLON-AEROGENES GROUP OF BACTERIA, WITH SPECIAL REFERENCE TO THE ORGANISMS OCCURRING IN THE SOIL

CHEN CHONG CHEN AND LEO F. RETTGER

From the Sheffield Laboratory of Bacteriology, Yale University

Received for publication December 10, 1919

From the time of the discovery of *Bacterium coli* and *Bacterium aerogenes* by Escherich this group of bacteria has been of profound interest to bacteriologists and sanitarians. As these organisms were first isolated from the human intestine, their presence elsewhere has generally been taken as an index of fecal pollution. They were later found, however, to be normal inhabitants of the alimentary tract of lower animals covering a wide range of species. The problem became still more complicated when it became known that coli-like organisms are widely distributed in nature, particularly in soil and on plants and cereals.

For a time it appeared as if the presence of an organism of the *B. coli* type in water or any other article of human consumption was of no sanitary significance. The subject was so confusing that one German school of sanitarians entirely discarded the colon test. In spite of this situation, the test for *B. coli* has been, and is today, strongly advocated and supported by sanitarians in America, England and France.

Numerous attempts have been made to devise an acceptable system of classification of the colon-aerogenes group, but with very few exceptions these have failed because they did not rest on a sound basis of natural relationships. In the light of recent researches it becomes quite apparent that difficulties in previous classifications were in large measure due to a lack of delicate and exact methods, neglect in the consideration of normal habitat, and faulty interpretation of results. New methods of study have aroused new interest in this group, however, so that within a

brief period of but a few years most important advances in our knowledge have been made. Some of the methods of earlier investigators have given place to newer procedures which rest on broader scientific foundations, as for example the differentiation of types by exact methods of determining the hydrogen ion concentration in media of known composition, and the quantitative relationship of carbon dioxide to the total gas volume. Again, older methods which had been practically discarded have been re-applied and made to serve as very important differentiating tests, as for example the Voges and Proskauer reaction.

In view of the claims held by many investigators that certain types of colon bacilli met with in water have their origin in soil, their occurrence in water being viewed only as the result of soil washings, etc., the question of relationship of types becomes all the more important. Correlation of characters with source, according to such claims, is a problem which requires a most careful and thorough solution, and if true correlation can be established, careful colon bacillus typing will be an essential part of sanitary water analysis.

Both *B. coli* and *B. aerogenes* were long regarded as being of fecal origin. Booker (1891), Hammerl (1897), Hellström (1901), McConkey (1905 and 1909), Clemesha (1912), Rogers and his co-workers (1914–16), Levine (1916), and others showed that the aerogenes type is relatively infrequent in human and animal dejecta, as compared with *B. coli* itself. Winslow and Cohen (1918) estimate the frequency of *B. aerogenes* in animal feces as 0 to 2.6 per cent.

The aerogenes type of colon bacilli appears to be widely distributed in nature, having as a rule a saprophytic existence. Laurent (1899) thought that *B. coli* may lead a parasitic existence on the potato. Klein and Houston a little later (1899–1900) reported the occurrence of both typical and atypical *B. coli* on grains of various kinds. Papasotiriu (1901) obtained results similar to those of Klein and Houston. Prescott (1902 and 1906) showed that coli-like organisms could be found on grains whose contamination with fecal matter seemed very remote. Düggeli

(1904) obtained a large number of similar bacteria from fruits, plants and seeds, and Metcalf (1905) observed the same kind of organisms on flowers, fruits and grains. Bettencourt and Borges (1908) found 12 strains of lactose-fermenting organisms on vegetables and cereals, but only half of these were typical *B. coli*.

McConkey found atypical colon bacilli to constitute 56.2 per cent of the 121 strains isolated from raw water. Houston showed that 13 per cent of his 243 strains from raw water, 5.3 per cent from stored water and 3.2 per cent from stored and filtered water, were atypical *B. coli*. More recently Rogers and his associates (1915–1916) found about 91 per cent of the bacteria on grains, and 33.3 per cent of those in water to belong to the group of non-fecal coli-like bacteria. Their investigations were soon followed by those of Rogers, Clark, etc., Hulton, Greenfield, Levine, Burton and Rettger, Winslow and Kligler, and others.

Houston (1897–1898), studied a large number of soil samples, and came to the important conclusion that true colon bacilli are rarely found in virgin soil, but are present in large numbers in soils that have been grossly polluted with animal matter. Konrich (1910) drew the same conclusions from his examination of 547 samples of soils. Both of these investigators failed, however, to differentiate typical *B. coli* from the non-fecal type (aerogenes-cloacae). Johnson and Levine (1917) found that the aerogenes-cloacae types are the predominant bacteria of this group in the soil. Burton and Rettger (1917) examined 1000 samples of soil, leaves, flowers, etc., and concluded that the cloacae type predominated over its close allies, which is in harmony with the results of Clemesha, who observed that this organism could be isolated readily from soils.

Conflicting reports on the distribution of coli-like bacteria in nature have appeared, however. Clark and Gage (1903) obtained negative tests with grains. Gordon (1904) was unable to isolate lactose fermenters from bran except that of inferior quality. Winslow and Walker (1907) failed to find them in a thorough search in 178 samples of grains and 40 samples of grasses. Neumann (1910) experienced the same difficulty with fresh fruits, but found them on fruits and others foods which had been exposed to human contamination.

The present investigation was planned with the following points in view:

1. To determine the relative frequency of the colon and aerogenes types of bacteria in soils which from all appearances are free from animal pollution.

2. To ascertain whether or not there is a definite correlation between types of bacteria and their origin.

3. To make an extended correlation study of the coli and the aerogenes types of gas-fermenting organisms with reference to some of the most important reactions, particularly the gas ratio, and the methyl red, Voges and Proskauer, and the uric acid tests; and to determine the relative value of these tests in identification work.

4. To determine the relative value of the following media in the present correlation study: (a) The dipotassium phosphate-glucose-peptone (Witte) medium of Clark and Lubs; (b) the same medium with American brands of peptone in place of the Witte; and (c) the synthetic medium of Clark and Lubs.

5. To ascertain whether the coli and aerogenes types of bacteria change their character under prolonged cultivation in a new environment.

METHOD OF COLLECTING SAMPLES

The samples were collected from four main sources in the vicinity of New Haven, Conn., at different seasons and over a period of two years. These sources are East Rock, West Rock, Mt. Carmel and the New Haven Water-shed. In most instances the samples were taken on the summits of the rocks or hills, and in places far remote from human habitations. Chance contamination from birds and wild animals cannot be excluded, however, except perhaps when the samples were taken from underneath precipices and rocks. Thorough sanitary surveys were always made of the surroundings.

Three types of soil were chosen: (1) those from open precipices, (2) from between and under large stones, and (3) from well-aerated open ground. They were selected in such a way as to minimize as far as possible all chances of contamination.

The collecting outfit consisted of a large test tube and a strip of tin one end of which was hammered into a spoon shape. The tin was about an inch longer than the tube, in which it was kept until the time of sampling, both being protected by a cotton plug. This outfit was sterilized in the hot air sterilizer. In taking a sample of soil the dry surface layers were usually removed by a sterile knife and 10 to 15 grams of the soil collected in the tube by means of the tin spatula. The samples were taken to the laboratory immediately.

Four different amounts of each sample were employed in the isolation process, namely 0.01, 0.1, 0.5 and 1.0 gram portions. At first these amounts were weighed out separately in watch glasses, but subsequently they were approximated by comparison with portions of known weight which were used as standards. The different portions of soil were vigorously shaken with definite amounts of water in dilution bottles. The shaking was continued until a uniform emulsion was obtained. After standing long enough for the heavy particles to settle out the supernatant fluid was drawn off and plated on plain agar. The direct plating method for isolation was employed throughout the investigation, in preference to the liquid sugar medium enrichment method. Although the latter method has often been employed, it was feared that its use would disturb the original numerical relationships of the bacterial types.

Litmus lactose agar was at first used in the plating, but was soon found to be very unsatisfactory. Very few red colonies were observed on the plates, and at times not a single red colony was discernable in as many as 20 to 30 plates after forty-eight hours of incubation. This was in harmony with Burton and Rettger's observations on the cloacae-aerogenes type of bacteria, and those of Ayers who showed that alkali-forming bacteria in milk would rarely be noticed on litmus lactose agar.

The plain agar plates were incubated at 30°C. for forty-eight hours. At the end of this period all of the coli-like colonies, noted on examination with the low power objective, were subcultured in lactose fermentation tubes. These tubes were incubated at 30°C. from two to five days, at the end of which time

a sorting out process was conducted. All tubes which failed to show gas production by the end of the fifth day were discarded. The tubes which contained gas were further plated on plain agar, for the purpose of more complete isolation or verification of purity of the cultures. Stock cultures were finally made from well-isolated colonies.

SOURCES OF SAMPLES		NUMBER OF SAMPLES	NUMBER OF SAMPLES YIELDING COLI-LIKE COLONIES	NUMBER OF CULTURES FROM POSITIVE SAMPLES	
				Aerogenes type	Coli type
Soils					
East Rock soil	A	23	7	29	1
	B	25	11	22	1
	C	14	5	20	1
	D	29	13	26	12
West Rock soil	A	23	6	31	0
	B	15	7	13	0
	C	18	11	29	0
	D	8	5	23	5
Water-shed soil	A	19	9	37	0
	B	28	15	40	0
	C	11	4	19	0
Mount Carmel soil	A	12	9	36	0
	B	15	3	37	0
	C	26	6	24	0
	D	6	4	14	0
	E	19	5	27	0
	F	20	7	20	0
Total		317	127	447	20
Feces					
Human (7)		7	7	0	41
Monkeys (2)		2	2	0	30
Horses (4)		4	3	0	31
Cows (3)		3	2	0	23
Sheep (4)		4	4	0	24
Fowls (3)		3	2	0	24
Total		23	20	0	173

For an organism to be admitted into the final collection of strains the following recognized characteristics were required. They must be short rods, staining readily with the ordinary dyes but not by the Gram method. They must fall in the class of non-spore-forming organisms, and possess the ability to attack lactose with the formation of acid and gas.

Altogether 467 strains were obtained from the various soil samples. Of this number 447 were finally identified as belonging to the aerogenes-cloacae subgroup or type, while the remaining 20 were designated as typical *B. coli*.

For comparative study the same types of organisms were sought in human and animal feces, and 173 coli-like organisms were isolated from the feces of 7 men, 2 monkeys, 4 horses, 3 cows, 4 sheep and 3 fowls. No organisms of the cloacae-aerogenes type were found, all of the isolated strains proving to be typical *B. coli*. This should not be regarded as evidence that the aerogenes-cloacae type is not present in the intestines of man and animals. It does indicate, however, that they are of uncommon occurrence there.

The table on page 258 contains a summary of all the samples taken from soil and human and animal feces, showing the number of strains obtained and the types of organisms isolated from each source.

MORPHOLOGY, STAINING PROPERTIES AND MOTILITY

When grown on plain agar the large majority of the strains were alike within narrow limits, and resembled in size and form ordinary *B. coli*, being distinctly rod-shaped, with rounded ends. Some strains were thicker than others, and frequently short forms were observed which were more or less coccus-like. All strains were Gram-negative, although some took the counterstain more deeply than others. All took the ordinary stains readily. Spore formation could at no time be demonstrated.

Motility studies were made on twenty-four hour cultures, grown in Clark and Lubs' medium, and for the greater part of the time by the hanging drop method. The Hesse method of cultivation in semi-solid agar was also employed. While in

many instances motility could be detected without any difficulty, it was at times very difficult to reach a satisfactory conclusion. The following table summarizes the results.

Motility

TYPES OF ORGANISMS	HANGING DROP METHOD		HESSE METHOD		NUMBER EXAMINED
	Motile	Non-motile	Motile	Non-motile	
B. coli	119	54	121	52	173
B. aerogenes	122	325	75	372	447

ACTION ON CASEIN, GELATIN LIQUEFACTION AND INDOL PRODUCTION

The reaction of the different strains in litmus milk was more or less uniform at the end of the observation period, namely seventy-two hours. Some difference was observed, of course, in the rapidity with which visible acid production was brought about; but with few exceptions all of the strains turned the litmus red and coagulated the casein within seventy-two hours. In the exceptional instances application of heat was necessary to bring about the coagulation.

Gelatin liquefaction was determined by the two following methods. In the first, tubes of nutrient gelatin were inoculated (stabbed) with a twenty-four hour agar culture and incubated at 20°C. for six weeks. Observations were made at the end of 3, 7, 14, 21, 39 and 42 days. In the second method, a loopful of a twenty-four-hour broth culture was spread over the surface of standard nutrient gelatin and incubated at 37°C. for thirty days. At the end of five-day intervals the tubes were placed in the refrigerator. Tubes in which no liquefaction was demonstrable were returned to the incubator. The results obtained by both methods are given in the following table.

The superiority of the 37° method is clearly brought out here. According to this, 17 of the aerogenes strains were liquefiers. None of the coli cultures possessed this property.

Gelatin liquefaction

ORGANISMS TESTED	20°C.		37°C.		NUMBER EXAMINED
	+	−	+	−	
B. aerogenes from soil	0	447	17	430	447
B. coli from soil	0	20	0	20	20
B. coli from feces	0	173	Not tested		173

The tests for indol were made by the Salkowski and by the para-dimethyl-amido-benzaldehyde method of Ehrlich (1901). In the former procedure 0.5 cc. of a 10 per cent solution of sulphuric acid and 0.5 cc. of a 0.01 per cent solution of potassium nitrite were added to 5 cc. of a five-day old culture which had been incubated at 30°C. The Ehrlich test was conducted by adding to the same amount of culture material 0.5 cc. of a 2 per cent solution of para-dimethyl-amido-benzaldehyde in 95 per cent alcohol and then concentrated hydrochloric acid drop by drop and in such a way as to keep the two layers of fluid separate. The medium employed in both tests consisted of 10 grams of Witte's peptone, 5 grams of sodium chloride, 0.2 grams of dipotassium phosphate, and 1000 cc. of water. The results of these tests are given in the following table.

Indol production

ORGANISMS	EHRLICH METHOD		SALKOWSKI METHOD		NUMBER OF STRAINS TESTED
	+	−	+	−	
B. aerogenes from soil	141	306	80	367	447
B. coli from feces	173	0	Not tested		173
B. coli from soil	15	5	15	5	20

The marked difference between the results obtained by the two methods with the aerogenes strains from soil is noteworthy, and is due either to the extreme sensitiveness of the Ehrlich reagent, or to the fact that the Salkowski method is in reality not an indicator of indol, but of some other reacting substance, as has been claimed by Kligler (1914) and others. All of the fecal strains of *B. coli* were indol-positive.

CARBOHYDRATE FERMENTATION

A. Acid production

The production of acids and gas by intestinal bacteria was observed by Buchner as early as 1885. He found that the main end products of sugar decomposition by his "Darmbacillus G" were carbon dioxide and fatty acids. Since this time the fermentation reaction in a culture medium has been extensively utilized for the purpose of characterization and classification of bacteria. Its principles have been so firmly established that it is considered one of the very important reactions in the differential studies of organisms of more or less similar as well as widely-divergent types.

At first the acid test was limited to a narrow field, namely, the separation of the colon from the typhoid type of bacteria, as is well illustrated in the early use of the litmus-lactose agar of Wurtz (1892); but at the present time no systematic study of an organism is complete without a resumé of its fermentative properties if it is found to have such. Our chief interest in this paper is, of course, in the colon-aerogenes group of bacteria.

In 1893 Theobald Smith separated the colon bacilli into two groups, according to their action on sucrose. The work of Durham (1900), however, inaugurated the real beginning of the use of the fermentation method in systematic classification. He employed glucose, lactose and sucrose, and characterized *B. communis verus* as glucoso-lactoso-non-sucroso fractor, *B. coli communior* as glucoso-lactoso-sucroso fractor, and *B. lactis aerogenes* as polysaccharid fractor. Ford (1901) chose the same sugars for his classification. He divided the colon group into *B. coli*, *B. lactis aerogenes*, and *B. cloacae*.

McConkey (1905) divided the lactose-fermenting group into the four well-known divisions, based principally upon the action on sucrose and dulcitol, namely: (1) sucrose – dulcitol – (*B. acidi lactici* type), (2) sucrose – dulcitol + (*B. communis* type), (3) sucrose + dulcitol + (*B. communior* type), and sucrose + - dulcitol – (*B. aerogenes* type). This classification for a long time served as a frame-work for investigators in this field. Wins-

low and Walker (1907) found that raffinose is generally attacked by sucrose-positive organisms.

Bergey and Deehan (1908) also adopted McConkey's classification, and by adding other cultural tests extended the grouping in a most bewildering manner. They derived 16 groups and 256 varieties. In 1909 McConkey again subdivided his four main groups by introducing more fermentable substances. Over 100 types were separated out in this way.

Jackson's classification (1911) was a modification of McConkey's four primary divisions. He divided these into 16 distinct types by the additional use of raffinose and mannitol. This was formally adopted in 1912 by the Committee on Standard Methods.

Rogers, Clark and Evans (1914) concluded from their study of the colon-aerogenes organisms in milk and milk products that the acid production from a fermentable substance can not be used to advantage. They claimed that the measurement of the gas ratio is far more reliable and constant than the titratable acidity. In his careful study of the acid production of the colon group in many different sugars and under varying experimental conditions Browne (1914) came to the conclusion that the degree of acidity produced by the various members of the colon group is directly proportional to the complexity of the sugar fermented, and that each type within the group has its own limit of acid toleration. One interesting fact brought out is that strains of colon bacilli isolated from feces produced more acid in fermentable sugars than those which were isolated from oysters.

Kligler (1914) studied 80 laboratory strains of *B. coli* and concluded that salicin fermentation offers a better basis of classification than dulcitol. He thus modified McConkey's scheme as follows: Sucrose − salicin + type (*B. communis*), sucrose + salicin − type (*B. communior*), sucrose + salicin, + (*B. aerogenes*) and sucrose − salicin-(*B. acidi-lactici*). Glycerol was further used, to differentiate *B. aerogenes* from *B. cloacae*, the latter being a non-glycerol fermenter.

Rogers, Clark and Evans (1915) again maintained that "the acid from the fermentation of sugar may be masked by a second-

ary alkali production sufficient in some cases entirely to obscure the acid formation." They, furthermore, discredited the salicin test on the ground that too large a percentage of their own cultures (94.6 per cent) fermented this substance.

Levine (1916) maintained that the origin of a strain correlates better with sucrose than with the sucrose-dulcitol fermentation. In his second paper (1916) he concluded that "quantitative acid production in glucose, galactose, maltose, lactose, sucrose, raffinose, salicin, inulin, mannitol, dulcitol and glycerol is not a reliable index for differentiating colon-bacillus-like bacteria." He believes that gas formation is of more value in classification.

Murray (1916) attempted to differentiate human, bovine and equine types of colon bacilli by means of quantitative acid production. He came to the same conclusions as Levine. "In all cases," he says, "the average acid production for each of the 100 strains of each type resembles that of every other, and also resembles the average acid production of all the strains taken together irrespective of origin."

Hulton (1916) studied 45 strains of colon bacilli from various sources and found that a better correlation was obtained between sucrose fermentation and source than between sucrose-dulcitol fermentation and source, confirming Levine's observations.

It appears very clear, from the above cited observations, that acid production, even when determined by the biometric method, does not furnish a sound basis for the classification of the colon-aerogenes group of bacteria. The relationship between the various cultural and physiological characters and the normal habitat of the organisms studied has been in the past lamentably neglected. This important phase of classification has recently been brought to light, however, by Rogers (1914–1916) and his associates. The grouping of the colon bacilli has by them been greatly simplified and placed upon a more natural and logical foundation. In their papers dealing with the bacteria isolated from milk and milk products, feces and grains, they gave us a sound criterion for the separation of the colon group into two distinct types. This separation was accomplished by the determination of the gas ratio in a glucose medium.

The fallacy of the titrimetric method of determining the amount of acid production by bacteria, and the necessity of the application of more accurate and dependable methods for estimating the H ion concentration in culture media and in bacterial cultures have been clearly demonstrated by Clark and by Clark and Lubs (1915–1917). As a result of their important observations, the colorometric method which has been so advantageously applied by others to biochemical problems in animal physiology has quite generally become a routine part of bacteriological procedure.

B. Hydrogen ion concentration and the methyl red test

The successful attempts of recent years to establish more definite relationships of the important members of the colon group with each other and to place their classification on a more scientific basis were stimulated by the works of Harden (1901 and 1905), Harden and Walpole (1906), and Thompson (1911). They found that the end products of glucose fermentation, such as lactic, succinic, acetic and formic acids, and ethyl alcohol, by *B. coli* differ in quantity from those which are formed by the organisms now known as *B. aerogenes* and *B. cloacae*. Michaelis and Marcora (1912) gave further evidence to show that there is a "physiological constant" in *B. coli* cultures when this type of organism is grown in lactose broth. This constant is evidenced by the cessation of its activity at a definite hydrogen ion concentration of 1×10^{-5}.

The Clark and Lubs (1915) phosphate-glucose-peptone medium serves admirably to apply the above principles to practical use. This, as well as the subsequent synthetic phthalate medium of these investigators, furnishes the necessary conditions to permit an indicator of the right choice to register the hydrogen ion concentration within sharply defined limits, so that the distinguishing character of the indicator is not altered or obscured. Their selection of methyl red as the indicator of merit has also done much to facilitate our study of these organisms.

Clark and Lubs found that *B. coli* produced definite changes in these media, with sufficient acid production to give a brilliant red coloration when a few drops of the methyl red indicator are added, whereas a similar test on cultures of the *B. aerogenes* type resulted in a yellow coloration. More than this, they showed that these reactions correlate in a perfect manner with the source of the strains, and with their gas ratio. These observations were soon followed by fruitful researches of other investigators, and were in a large measure corroborated by Levine, Winslow and Kligler, Greenfield, Fulton, Johnson, Burton and Rettger, and Winslow and Cohen.

There are, however points still undetermined in the methyl red test which require further elucidation. Clark and Lubs emphatically state that the use of peptone other than Witte's would lead to an erroneous reaction. With this brand of peptone there are doubtful reactions which have been termed by Clark and Lubs as the "neutral tints." The typing of bacteria by means of the methyl red test depends upon gradations in color varying from a yellow to a bright red color, and covering a range of 4.2 to 6.0 on the pH scale. Determining the hydrogen ion concentration is comparatively easy when pronounced shades of red are obtained on the addition of the indicator; but when the hydrogen ion concentration is so low that only a yellow color ("neutral tints") is produced by the methyl red, it is imperative that some sort of a scale or limit within the "neutral tints" be established, or the types may be incorrectly placed and the correlations disturbed. Levine (1916) in his first work on the methyl red test encountered this difficulty; and Burton and Rettger (1917) found that the variation in the high ratio cultures was such as to lead them to the conclusion that the biometric method was of little value in the classification. Winslow and Cohen (1918) encountered this difficulty in the Witte peptone-phosphate-glucose medium, as well as in the synthetic medium later devised by Clark and Lubs (1916).

EXPERIMENTAL

In the present investigation the methyl red test has been placed on a more strictly quantitative basis, and exact pH values have been given to the various shades of color that were obtained at different times, both in the "neutral tint" range as well as where the characteristic light rose to deep red colors occurred.

In view of the fact that the inconsistent results obtained by some investigators were attributed by some to the indiscriminate use of different brands of peptone, two well-known products were employed, namely Witte's and Difco. The composition of the medium was the same as that first recommended by Clark and Lubs (0.5 per cent each of di-potassium phosphate, peptone and glucose). The new synthetic medium of Clark and Lubs was also used, for comparison.

No definite standards as to the temperature and incubation time for the methyl red test seems thus far to have been agreed upon by different workers. Clark and Lubs chose 30°C. and an incubation period of three to five days, whereas Levine maintained that no difference could be observed whether the temperature was 30 or 37°C. He held that 3 days' incubation is sufficiently long if the temperature is 37°C. More recently, Johnson and Levine (1916) resorted to forty-eight hours' incubation at 37°; Burton and Rettger (1917), five days at 37°; and Winslow and Cohen (1918), four days at 37°C.

Preliminary studies showed that a large number of the soil organisms refused, at least for a while, to grow at 37°C., especially in the synthetic medium, thus apparently confirming the findings of Rogers and his co-workers, and those of Winslow and Cohen. Throughout the present study the incubation temperature was 30°C. Since there was still a question as to the most desirable incubation period to be employed in order to obtain the most uniform and dependable results, both a three and a five day period were given a lengthy trial with all three of the media.

The synthetic medium had a distinct advantage over the others in being practically if not entirely colorless after sterilization, and hence not obscuring the color reactions. However,

after some preliminary attempts to obtain colorless peptone media, a method was followed by which the peptone-phosphate-glucose medium could be prepared and sterilized so that it remained as free from color as the synthetic. Previously sterilized test tubes are filled with the required amount of medium, and the final sterilization carried out by placing the tubes in containers which have impervious bottoms and sides, as for example canning tins or glass beakers, instead of the usual wire baskets, and sterilizing in the autoclave for ten minutes at an extra pressure of 10 pounds. During the entire course of the investigation very few tubes were found not to be sterile. We made it a common practice not to employ the tubed medium until its sterility had been tested by proper incubation for at least twenty-four hours.

The colorimetric standards were prepared according to the descriptions of Clark and Lubs. Three color indicators were employed, namely methyl red, brom thymol blue and brom cresol purple. Brom thymol blue is particularly well adapted for registering the hydrogen ion concentration of aerogenes cultures. The brom cresol purple was selected because it gives more distinguishable shades of color than the methyl red at the less acid end of the methyl red range where the troublesome neutral tints occur. The following technique was adopted for the test.

Five cubic centimeters of the bacterial culture fluid were transferred to test tubes of uniform diameter, and 0.2 cc. of the indicator added which by the preliminary drop method on a watch glass, or better still on a sheet of glazed paper or cardboard, was found to cover the indicated hydrogen ion range. The tubes were sorted out into three groups, with a fair knowledge of the approximate range of hydrogen ion concentration of each group. The pH value of each culture was recorded by comparison in the comparator with the previously prepared color standards.

A total of 3725 individual hydrogen ion concentration determinations was made in the three media with the two types (coli and aerogenes) isolated from soils and feces. The results are shown in the accompanying charts, in which the frequencies of occurrence are plotted as ordinates, and the corresponding pH values as abscissae. The middle line in the charts is an arbitrary

border or methyl red line to separate the coli from the aerogenes types of organisms. Since the colon type is now accepted as methyl red positive, and since the pH value 6.0 is the end point of the methyl red range, this line which starts at pH 6.0 appears to be the natural line of demarcation between these two types, and to be of value in plotting the charts.

Chart I gives the results of the hydrogen ion concentration determinations of the 447 aerogenes strains grown in synthetic and Witte's peptone media for three days at 30°C. Both curves

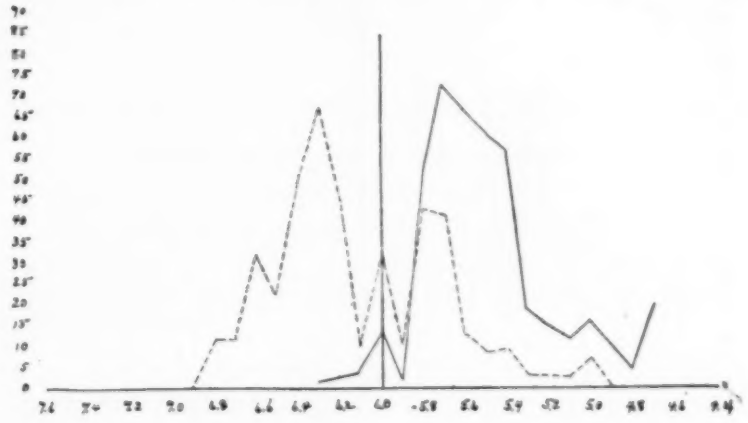


CHART 1. FREQUENCY CURVES FOR B. AEROGENES TYPE (SOIL)

Ordinate represents numbers of strains and abscissa the pH values. Three days' incubation at 30°C. Unbroken line, synthetic medium; broken line, peptone-glucose-phosphate medium (both of Clark and Lubs).

show clearly that a three day period of incubation is not sufficient at this temperature for the methyl red test, especially in the synthetic medium, in which almost all aerogenes cultures become methyl red positive. The peak of the curve for the synthetic medium is located at pH 5.7 and even with the clark medium, a large number of aerogenes cultures enter the methyl red range, although not as many, as in the synthetic medium.

Chart II shows the results of five days' incubation in the three media. There is a striking difference between the results of the

three and five-day periods. The additional forty-eight hours' incubation has returned all of the aerogenes strains to their proper indicator ranges in the synthetic as well as in the Witte peptone medium. The peak of the curve in the synthetic medium is now located at pH 6.5 instead of 5.7, the whole curve covering a pH scale of 6.0 to 6.8. In the Witte peptone medium the pH scale extends from about 6.0 to 7.6, whereas the peak lies at 6.8.

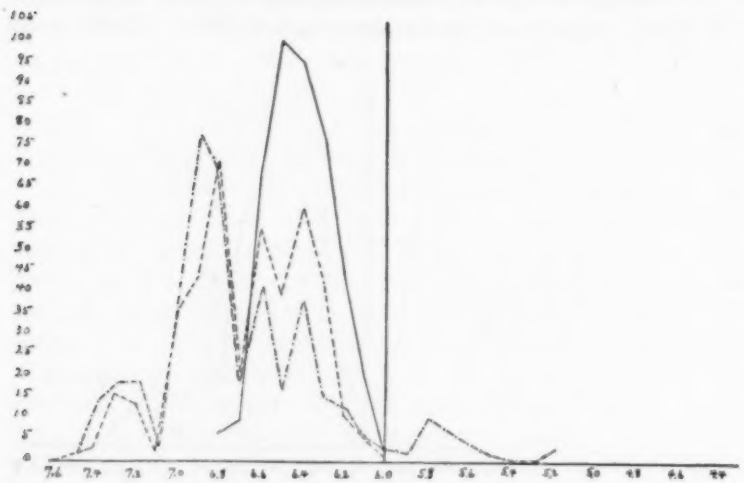


CHART II. AEROGENES TYPE (SOIL)

Five days' incubation at 30°C. Unbroken line, synthetic medium; barred line, Witte peptone-glucose-phosphate medium; barred and dotted line, Difco peptone-glucose-phosphate medium.

When substituting Difco peptone for the Witte in the peptone-phosphate-glucose medium, certain discrepancies occurred, even after prolonged incubation. It is seen that 26 of the aerogenes strains entered the methyl red positive range.

Chart III gives the results of 20 coli cultures isolated from soil, and grown in both the synthetic and the Witte peptone media for three days at 30°C. Chart IV gives the results of the same 20 coli strains in all three of the media after an incubation period of five days.

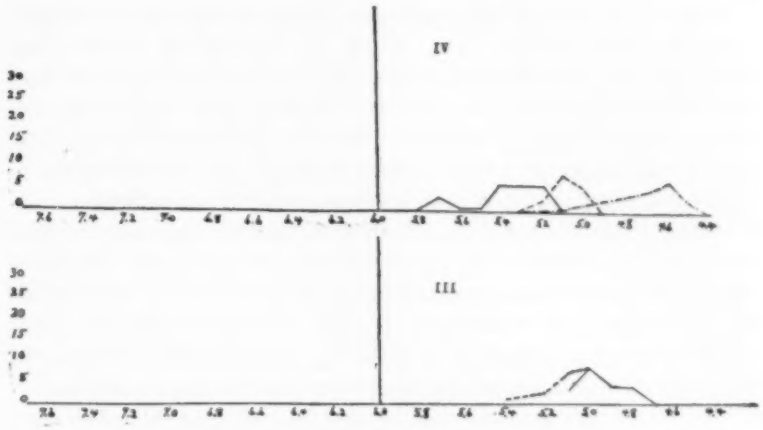


CHART III. (BELOW). B. COLI TYPE (SOIL)

Three days' incubation at 30°C.

CHART IV. (ABOVE). B. COLI TYPE (SOIL)

Five days' incubation at 30°C. Unbroken line, synthetic medium; barred line, Witte peptone-glucose-phosphate medium; barred and dotted line, Difco peptone-glucose-phosphate medium.

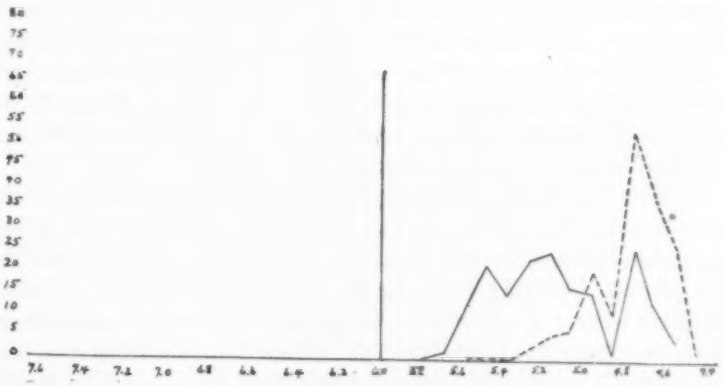


CHART V. B. COLI TYPE (ANIMAL SOURCE)

Five days' incubation at 30°C. Unbroken line, synthetic medium; broken line, Witte peptone-glucose-phosphate medium.

Chart V is plotted with two curves representing 173 coli strains isolated from animal feces. These are the results of the final hydrogen ion concentration determinations in the synthetic and the Witte peptone media after five days at 30°. Again the coli cultures gave uniform results with the methyl red tests. The pH values of these cultures cover a range of from 4.5 to 5.6 in the Witte peptone, and 4.6 to 5.8 in the synthetic medium.

Taking the three coli charts (III, IV and V) together, we find no qualitative differences in the results obtained with the three media or the two incubation periods, in so far as the methyl red test is concerned. This goes to show that incubation for three days at 30°C. is sufficient to give the characteristic methyl red reaction by coli strains from both fecal and non-fecal sources.

A careful analysis of these curves reveals several interesting facts. We find that the hydrogen ion concentration of the aerogenes cultures is generally higher in the synthetic than in the Witte peptone-glucose-phosphate medium either after a three or five days' incubation period. This would indicate that the production of alkalinity by aerogenes strains is more rapid in the Witte peptone medium than in the synthetic. In the former the peak of the H. I. C. curve for the three days' period is in the majority of cultures located at pH 6.3, while in the synthetic medium the peak lies at 5.7. A still more striking difference is shown in the five-day curves. In the synthetic medium the pH peak lies at 6.5, and the curve covers a range of only 6.0 to 6.8, whereas in the Witte peptone phosphate glucose medium the curve extends over a wider pH scale, 6.0 to 7.6, with the peak at 6.8.

In the Difco peptone medium the results are of peculiar interest. Setting aside for the present the few discrepancies (26 out of 447 aerogenes cultures which gave the methyl red positive test), a remarkable similarity between the Witte and the Difco peptone medium curves is observed. They almost parallel each other, from the range of pH 6.0 to 7.5. Their four peaks at pH 6.4, 6.6, 6.8 and 7.3, and the three troughs at 6.5, 6.7, and 7.1 correspond almost exactly.

REVERSION OF REACTION

The principle involved in the differentiation of the coli from the aerogenes type of bacteria by means of the Clark and Lubs media and the methyl red test is as follows. *B. coli* soon reaches a growth-inhibiting or lethal zone of hydrogen ion concentration and remains there; *B. aerogenes*, on the other hand, by virtue of its peculiar metabolic properties, continues its action upon the medium and progressively raises the pH value to a maximum which does not in itself retard further development of the organism. *B. coli* rapidly attacks the glucose, with relatively large acid production, while *B. aerogenes* produces more gas and correspondingly less acid during the early stages of growth than the coli type. *B. aerogenes* brings about a reversion of reaction which after prolonged incubation becomes more and more apparent.

This reversion of reaction in aerogenes cultures has led Kligler and a number of other investigators to assume that it is due to the production of ammonia from a peptone-glucose medium after exhaustion of the sugar. In their further study of the subject, Clark and Lubs disproved this view. They showed that reversion of reaction can take place in a synthetic medium free from peptone, and further proved that an extreme reversion can be obtained in a synthetic medium containing an ammonium salt as a source of nitrogen, although the content of total nitrogen is reduced to a point at which its participation in any form in changes of reaction of the medium would be insignificant. They come to the conclusion, therefore, that "An increase in ammonia may accompany the reversion of reaction, but the amount liberated is inadequate to account for the extent of the reversion." Under such conditions they believed that "It should not be assumed that the reversion is due solely to ammonia production."

These conclusions of Clark and Lubs do not give us a satisfactory explanation as to the cause of the reversion, nor do they lead us to think that they deny ammonia production in a glucose-peptone medium. It remained for Ayers and Rupp (1918) to furnish a plausible explanation for the phenomenon of reversion in a sugar-peptone medium. They demonstrated that there is

a simultaneous acid and alkaline fermentation in which the salts of organic acids produced from glucose are converted into carbonates or bi-carbonates, which in turn cause the reversion in reaction in a *B. aerogenes* culture.

In our attempts to follow up the work of Ayers and Rupp the Sörensen method of determining primary amino acid was employed, in addition to the ammonia determination method of Folin. It was hoped that some definite relationship between the formol titration and the ammonia figures could be established.

The Clark and Lubs peptone-phosphate-glucose medium was again made use of. Flasks containing 500 cc. of the medium were inoculated with a loopful (standard 4 mm. loop) of a twenty-four-hour culture of *B. aerogenes* grown in the same medium, and incubated at 30°C. At the end of 1, 2, 3, 5, and 10 day intervals the required amount of culture material was removed and subjected to the tests for sugar, amino acids, ammonia and hydrogen ion concentration. Eight representative strains of bacteria were selected for the test. Two were *B. coli* isolated from animal feces, and the remaining six aerogenes types from various apparently uncontaminated soils.

In the following table the Sörensen titration figures are recorded as the numbers of cubic centimeters of $\frac{N}{10}$ NaOH required to neutralize 100 cc. of the test medium. The ammonia figures are given as milligrams of ammonia per 100 cc. of the culture fluid.

The results show several interesting points. A close relationship appears to exist between the sugar utilization and the hydrogen ion concentration. In the aerogenes cultures the complete reversion of reaction took place within five days' incubation, and the sugar supply was practically exhausted in the same period. On the other hand, in the coli cultures the glucose was only partly fermented, and the hydrogen ion concentration remained constant after three days of incubation. No definite relationship between the Sörensen titration and the ammonia figures could be established. Irregularity of ammonia production is noticeable. One point stands out clearly from the rest; the amount of ammonia recorded in the *B. aerogenes* cultures was no greater, after ten days' incubation than in the control.

CULTURES		DAYS	pH	SÖRENSEN	AMMONIA	SUGAR
Control	Uninoculated		6.9	10.0	3.17	+
Aerogenes type	No. 34 Soil	1	5.2	10.0	2.20	+
		2	4.9	10.0	1.40	+
		3	4.9	20.0	1.70	+
		5	6.1	15.0	1.60	Trace
		10	6.9	10.0	2.10	Trace
	No. 37 Soil	1	5.4	20.0	2.75	+
		2	5.4	10.0	2.10	+
		3	6.1	10.0	1.92	Trace
		5	6.7	20.0	2.55	Trace
		10	6.8	15.0	2.01	Trace
	No. 24 Soil	1	5.9	10.0	1.40	+
		2	5.9	20.0	1.84	+
		3	6.3	15.0	2.50	Trace
		5	6.8	10.0	3.10	0
		10	6.8	10.0	2.54	Trace
	No. 44 Soil	1	5.9	15.0	1.45	+
		2	5.9	20.0	1.75	+
		3	6.3	10.0	2.04	0
		5	6.4	10.0	1.70	0
		10	6.5	35.0	2.80	0
	No. 20 Soil	1	5.9	20.0	1.50	+
		2	5.9	30.0	1.45	+
		3	6.3	20.0	1.78	Trace
		5	6.4	10.0	2.80	Trace
		10	6.8	20.0	3.02	Trace
	No. 21 Soil	1	5.1	20.0	1.64	+
		2	5.0	30.0	2.90	Trace
		3	5.3	10.0	3.10	Trace
		5	6.0	20.0	2.45	Trace
		10	6.5	30.0	2.70	0
Coli type	No. 5 Human feces	1	4.9	15.0	2.40	+
		2	4.6	20.0	3.30	+
		3	4.8	10.0	2.10	+
		5	4.7	10.0	3.10	+
		10	4.7	20.0	3.04	+
	No. 27 Human feces	1	4.8	10.0	1.40	+
		2	4.6	10.0	1.50	+
		3	4.7	15.0	2.90	+
		5	4.7	20.0	2.10	+
		10	4.7	15.0	3.12	+

The interpretation of the results is fraught with some difficulty. Clark and Lubs, and Ayers and Rupp did not deny that ammonia production may accompany the phenomenon of reversion. Kendall's theory of ammonia recession may in part explain a certain phase of reversion, and the claim of Rettger and Berman, and others, that ammonia is a readily available food for bacteria, and that it is not an end product, but an intermediary stage of metabolism, may throw considerable light on the subject.

GAS PRODUCTION

Escherich (1885) clearly demonstrated the production of gas in glucose and lactose media by his two types of intestinal organisms. Arloing confirmed the observation of Escherich. It was not until after the appearance of the fermentation tube (1890–93) of Theobald Smith, however, that the determination of gas volume and ratio formed part of our scheme of differentiation of bacteria. For almost twenty years this method reigned supreme and its accuracy as a quantitative procedure was little questioned.

Although Harden, Walpole and Thompson had improved the Smith method, the analytical error was not eliminated. Keyes (1909) introduced the vacuum method of exact gas analysis, and determined the gas ratio of *B. coli*. His work was followed by that of Rogers and his associates (1914–15), who firmly established the gas ratio of *B. coli* and *B. aerogenes*, namely approximately 1:1 (CO_2:H) for the former, and 2:1 for the aerogenes type. Since then there appears to be no doubt that the principle of gas-ratio as a differential test is well founded.

In the present study the gas production was at first observed in the Durham fermentation tube. A gas volume of at least 10 per cent was recorded as a positive test for gas, while the acid reaction in the same tubes was roughly determined with Bromcresol-purple, a distinct yellow color being recorded as a positive reaction. In the use of the Durham fermentation tube a long inner tube (3 inches) was employed to facilitate the reading of the gas volume, which was done by the Frost gasometer. Al-

though this method is very crude, some interesting data were obtained.

Cultures of the *B. coli* type were seldom observed to attain a gas volume greater than 40 per cent when the medium had 1 per cent of glucose, the amount of gas usually being between 20 and 40 per cent. More specifically, of 173 fecal strains of *B. coli* cultures used only 8 gave a gas volume of 40 per cent, all of the remaining 165 falling below this figure. On the other hand, 322 out of 447 aerogenes strains gave a percentage higher than 40, 74 produced 40, and only 51 gave less than 40 per cent.

On account of the lack of proper facilities gas determination by the exact method was not undertaken until toward the end of the present study. Considerable time was spent in designing a vacuum pump which was a modification of the Sprengel pump, the plan of the Boltwood pump being followed to a large extent. As the Boltwood pump is not adapted for the collection of gases, and as no provision is made for removing the minute air bubbles which collect in the mercury, it had to be further modified. The distinct features of the new design of vacuum pump are: (1) It can be employed for exhaustion of the gases as well as their collection, (2) It possesses a device for the removal of air bubbles from the mercury, and (3) Its operation is automatic and continuous. As it is planned to give a complete description of this apparatus in a separate publication (Chen), further discussion of its design and application is omitted here, except a brief allusion to the following table which gives the representative results of one of the few estimations which were hurriedly made at the close of the present study, and which as far as it goes confirms the gas ratio of Rogers, Clark, etc.

Gas-production in vacuum bulbs

TYPE	SOURCE	TOTAL GAS	RATIO			
			CO_2	H_2	CO_2/H_2	Residue
		cc.				
Aerogenes.....	Soil no. 7	20.80	7.83	3.62	2.16	9.35
Coli.....	Human feces No. 36	11.70	4.26	3.90	1.06	3.54

THE VOGES AND PROSKAUER REACTION

This reaction was first observed by Voges and Proskauer (1898) in their studies on the bacteria of hemorrhagic septicemia. It was soon found that certain members of the colon group gave the reaction, and that on this as a basis the typical and atypical, or the fecal and non-fecal, types coming under this group could be differentiated from each other. Durham (1901), McConkey (1905–09), Archibald (1907), Bergey and Deehan (1908), Ferreira, Horta and Paredes (1908), West (1909), Copeland and Hoover (1911), Clemesha (1912), Kligler (1914), Levine (1916), and more recently Hulton, Greenfield, Johnson, Winslow and Kligler, Burton and Rettger, Winslow and Cohen, and Rogers and his associates have made use of this reaction. As a result the test has been given added significance, and promises to be one of the most important methods of differentiating the coli from the aerogenes type of organisms.

The chemistry of this reaction was not known until the publication of the thorough and painstaking researches of Harden and his co-workers. Harden (1905) in his study of the fermentation of glucose by colon bacilli discovered two important facts concerning the end products of glucose fermentation of the coli and aerogenes types of bacteria. He found that *B. coli* gives a high acidity, with only a partial utilization of the glucose, whereas the aerogenes type produces low acidity and complete exhaustion of the sugar. He concluded that "*B. lactis aerogenes* acts upon glucose in a totally different manner from *B. coli* and is therefore to be regarded as a distinct organism."

Harden and Walpole (1905–06) recognized a new substance, glycol, among the usual products of glucose fermentation, such as lactic, acetic, formic and succinic acids, alcohol and carbon dioxide. According to them, this new substance, crude glycol, contains a large amount of 2:3 butylene glycol (CH_3-CHOH-CHOH-CH_3) which in the presence of atmospheric oxygen is oxidized to acetyl-methyl-carbinol (CH_3-CO-CHOH-CH_3). This volatile reducing substance is further oxidized in a relatively strong alkaline solution to di-acetyl (CH_3CO-CO-CH_3). The

diacetyl is colorless as such, but when brought into contact with a small amount of peptone or other protein material produces the characteristic eosin-like red coloration.

Walpole (1910) further observed that by passing a current of air through the *B. aerogenes* culture five to six times as much acetyl-methyl-carbinol is produced as when the culture fluid is left undisturbed. Thompson (1911) found that the chemical action of *B. cloacae* of Jordan on glucose is practically the same as that of *B. aerogenes*, in so far as the production of carbinol is concerned.

The actual correlation of the Voges and Proskauer reaction with the source of the organism and with its other characteristics was the work of Levine (1916). Soon after Rogers and his coworkers announced the definite relationship between the gas ratio, gas volume and the hydrogen ion concentration of the coli and aerogenes types of bacteria, Levine showed that an added correlation could be established with the Voges and Proskauer reaction. He found that bacteria of the aerogenes type consistently gave a positive V and P reaction, whereas those which were positive to the methyl red test were regularly V and P negative.

In the present study an attempt was made to determine: first, the relative value of the three media previously described in this paper, namely the Clark and Lubs' Witte peptone-glucose-phosphate, the synthetic phthalate-glucose-phosphate, and the Difco peptone-glucose-phosphate medium, for the study of the Voges and Proskauer reaction; second, the most favorable period of incubation; and third, a rapid and practical method for carrying out the test.

In the original method of applying the Voges and Proskauer test the alkaline fluid was exposed to the atmosphere for twenty-four hours or longer at room temperature. Walpole tried to hasten the oxidation process by passing a current of air through the medium, while West, following "Test No. 1" of Revas, boiled his cultures. More recently Levine introduced various oxidizing agents, and Bunker, Tucker and Green (1918) advocated the use of Syracuse watch glasses. These methods, and particularly the last two, appear to us too uncertain and too laborious.

The technique finally adopted in this work was as follows: At the end of one, three and five days' incubation periods 5 to 6 cc. of the culture fluid were well shaken in the test tube with an equal amount of 10 per cent potassium hydroxide solution. The tubes were placed in an incubator having a constant temperature of 30°C. At the end of one to three hours the tubes were again vigorously shaken until the liquid became foamy. As a rule, the eosin-like coloration made its appearance quickly, and the color deepened throughout the tube, instead of becoming visible as a ring or a surface layer. Without an exception, a decidedly positive Voges and Proskauer reaction (maximum color production) could be observed within a few hours (two to three) by this method. West used a more or less similar shake method ten years ago. He said: "After heating the tube, shake well, or blow through it to bring the red color out."

By the use of the method just described a total of 3725 individual Voges and Proskauer tests were made in the three different glucose-phosphate media, following three different incubation periods. The results are shown in the following chart (VI).

The chart shows clearly that there was no difference in the color reaction as the result of differences in the length of the incubation periods, one, three and five days, or of the different brands of peptone employed. Although the coloration in the synthetic medium was not so uniformly strong as in the peptone media, the reaction was always sufficiently pronounced to be called distinctly positive

Different strains manifested a striking difference in the degree of coloration which they gave. Not a single aerogenes culture, however, failed to give the reaction. On the other hand, not one of the colon strains, either from soil or feces, responded to the test.

An attempt was made further to determine the minimum incubation period in which color production can be obtained. A small collection of typical and representative strains of *B. aerogenes* was tested in the same three media after four, ten and fourteen hours of incubation of the cultures at 30°C. The results showed that a positive Voges and Proskauer reaction may be ob-

CHART VI. Upper section, showing Voges and Proskauer reaction in very young cultures of B. aerogenes. Lower, main section, showing V. & P. reactions in the different media after one, three and five days' incubation at 30°C. C = Coli type; A = aerogenes type. The figures below indicate number of strains. Shaded areas show weak reactions.

tained in cultures which are only ten to fourteen hours old (chart VI, upper part).

THE DEPORTMENT OF MIXED CULTURES (COLI AND AEROGENES TYPES) TOWARD THE METHYL RED TEST AND THE VOGES AND PROSKAUER REACTION

One strain of typical *B. coli* of fecal origin was grown in symbiosis with 56 different strains of *B. aerogenes* obtained from soil. Each of the strains had been cultured separately in plain broth for twenty-four hours, and a loopful of each tube was inoculated into the Witte peptone- and the synthetic medium of Clark and Lubs. The mixed cultures were incubated for five days, one set at 24° and a second at 37°C. The hydrogen ion concentration was then determined with methyl red as indicator, and the Voges and Proskauer test was made as described earlier in this paper.

The results of the study are given in the accompanying chart (VII). They show that in the peptone-phosphate-glucose medium, both at 24° and at 37°, the hydrogen ion concentration of the aerogenes type of organism was partly disturbed, and that in the synthetic medium the acidity of the colon type almost entirely obscured that of *B. aerogenes*. The Voges and Proskauer reaction as ordinarily given by the aerogenes type was not at all affected in the synthetic medium at 24°, and only one culture in the peptone medium failed to give the characteristic reaction when grown at 24°C. Of the 56 tubes incubated at 37°, 14 were negative by the V and P test, 4 in the synthetic and 10 in the peptone medium.

Briefly stated, when both types of organisms are present in any given culture the methyl red test reveals the presence of the colon type, while the aerogenes type responds to the Voges and Proskauer test when the temperature of incubation has been as low as 24°. At the higher temperature only a small number of the cultures are negative to the V & P test. The permanency of the Voges and Proskauer reaction is here clearly demonstrated.

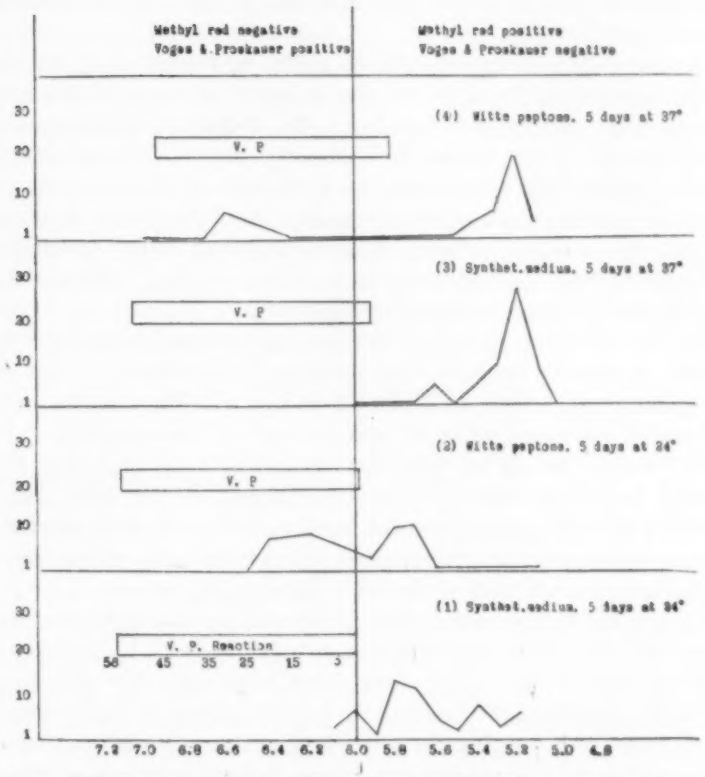

Methyl red negative
Voges & Proskauer positive
Methyl red positive
Voges & Proskauer negative
(4) Witte peptone. 5 days at 37°
V. P
(3) Synthet. medium. 5 days at 37°
V. P
(2) Witte peptone. 5 days at 24°
V. P
(1) Synthet. medium. 5 days at 24°
V. P. Reaction
50 45 35 25 15 5
30
20
10
1
7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8

CHART VII. SHOWING SYMBIOTIC REACTIONS OF ONE STRAIN OF B. COLI WITH 56 STRAINS OF B. AEROGENES

DIFFERENCES IN NITROGEN UTILIZATION AS A BASIS FOR DISTINGUISHING B. COLI FROM B. AEROGENES. THE URIC ACID TEST OF KOSER

The selective action of bacteria on carbohydrates has long been recognized, and on this principle many tests have been devised, and different types of bacteria have been successfully separated. The differentiation rests, however, on the character of the end products of carbohydrate fermentation, as for example

the formation of acid and gas, and the production of carbinol and diacetyl.

Much attention has also been given in recent years to the differentiation of bacteria on the basis of nitrogen utilization, in the form of ammonia, amino acids, di-amines, nitrates, etc. For example, it was shown by Uschinsky that *B. coli* can attack amino acids like asparagine in a purely synthetic medium, whereas *B. typhosus* is as a rule unable to utilize it as a source of nitrogen. Quite recently Koser (1918 and 1919) succeeded in a somewhat similar manner in differentiating between the *B. coli* and *B. aerogenes* types of organisms.

In his extensive study of nitrogen utilization by bacteria in media of definite chemical composition, Koser observed that *B. aerogenes* readily attacks uric acid or hypoxanthine as the only source of nitrogen, while *B. coli* is void of this property. In other words, he found that the aerogenes type possesses the unique power of seizing upon the nitrogen of the purin ring, whereas the coli type leaves this portion of the uric acid molecule intact, and fails in its development unless some other nitrogenous substance which furnishes available nitrogen is present.

In the present investigation all of the strains isolated from feces and soils were employed in a further study of this phase of bacterial nutrition. They were inoculated into the uric acid synthetic medium of Koser. In a large series of tests other purin bases were employed in the place of the uric acid, the basic composition of the synthetic medium being the same as when the uric acid was used. These substances were xanthine, caffein and theobromin. The uric acid synthetic medium as prepared by Koser has the following composition:

Distilled ammonia-free water	1000.0 cc.
NaCl	5.0 grams
$MgSO_4$	0.2 gram
$CaCl_2$	0.1 gram
K_2HPO_4	0.1 gram
Glycerol	30.0 grams
Uric acid	0.5 gram

The medium was tubed in 5 cc. portions and sterilized for fifteen minutes at 12 pounds extra pressure. The test tubes

which are used must be scrupulously clean; small amounts of nitrogenous matter left on the walls may furnish sufficient nitrogen for the organisms to multiply so as to obscure the sharp distinction between the appearance of the *B. coli* and *B. aerogenes* tubes after the required time of incubation, four to five days at 30°C.

A twenty-four hours growth of the organism under observation was used as a rule as inoculum, and this was transferred with precaution, so as to limit as far as possible the amount of cell material and products of bacterial metabolism which are carried over. The results of the tests with the uric acid medium are summarized in the following table.

Table showing results of uric acid tests

	POSITIVE	NEGATIVE	TOTAL
Aerogenes strains from soils	447	0	447
Coli strains from feces	0	173	173
Coli strains from soils	10	10	20

All of the aerogenes strains (soil) gave a very pronounced growth in the uric acid medium, while none of the fecal strains of the coli type clouded the medium or in any way showed indications of development. The differentiation was in every instance sharp though the degree of turbidity varied much. However, considerable discrepancy was observed among the coli strains isolated from soils. Of the 20 cultures employed 10 were uric acid positive and 10 negative. In the tubes in which the uric acid was attacked the growth was as luxuriant as in typical cultures of the aerogenes type. Lack of correlation here of the 10 uric acid positive strains of soil coli with the methyl red test and Voges and Proskauer reaction may be of some significance as pointing to the possibility that these ten strains are of a type intermediate between the coli and the aerogenes.

The same results were obtained in the xanthine synthetic medium as in the uric acid, except with the soil strains of *B. coli*, which goes to show further that the failure of *B. coli* to utilize the nitrogen is due to its inability to disrupt the purin ring,

which contains the nitrogen. All of the soil coli strains failed to attack the xanthine, and hence reacted like typical *B. coli*. Further investigation may show that xanthine may be superior even to uric acid for differentiating these two types of bacteria, unless types intermediary between coli and aerogenes exist which in uric acid medium are sharply distinguished. With two exceptions, both types of bacteria failed to develop in the caffeine and the theobromine medium. The results with the xanthine, caffeine and theobromine media are shown in the following table.

Table giving results with xanthine, caffeine and theobromine media

ORGANISMS	XANTHINE		THEOBROMINE		CAFFEINE		TOTAL
	+	−	+	−	+	−	
Aerogenes (soils)	55	0	0	55	0	55	55
Coli (soils)	0	20	0	20	0	20	20
Coli (feces)	0	55	2	53	0	55	55
Aerogenes (Am. Mus. Nat. Hist.)	10	0	0	10	0	10	10
B. communior (Am. Mus. Nat. Hist.)	0	5	0	5	0	5	5
B. communis (Am. Mus. Nat. Hist.)	0	5	0	5	0	5	5
Aerogenes (Rogers)	6	0	0	6	0	6	6
Aerogenes (Winslow and Cohen)	5	0	0	5	0	5	5
Total							161

FERMENTATION OF GLUCOSE, LACTOSE, SUCROSE, ADONITOL AND DULCITOL

It was at first intended to classify the present collection of strains according to McConkey's primary divisions, on the basis of fermentation of glucose, lactose, sucrose and dulcitol. Adonitol was also employed because of the claims of Rogers and others that the so-called fecal and non-fecal types of *B. aerogenes* can be differentiated in this way. Owing to our inability to obtain sufficient dulcitol the classification study with McConkey's four sugars was incomplete. The results are presented in the following table.

BACTERIAL STRAINS	GLUCOSE		LACTOSE		SUCROSE		ADONITOL		DULCITOL		TOTAL
	+	−	+	−	+	−	+	−	+	−	
Aerogenes type (from soils)	447	0	447	0	338	109	152	295			447
Coli type (from soils).....	20	0	20	0	15	5	5	15			20
Coli type (from feces).....	173	0	173	0	91	82	30	143			173

Results with dulcitol too incomplete to include in table. The figures indicate both acid and gas production.

All of the strains fermented glucose and lactose. Sucrose was attacked by both the colon and aerogenes types, although the high gas ratio group was as a group more active than the other, excluding the soil coli.

The present results do not bear out the contention that the fecal and non-fecal types of aerogenes may be differentiated by fermentation in adonitol, as 152 soil aerogenes strains out of the 447 attacked the adonitol, whereas the remaining 295 did not. These findings agree in principle with those of Winslow and Cohen, who observed that "a greater proportion of *B. aerogenes* from the unpolluted sources attacked adonité than did those from the polluted waters."

ATYPICAL STRAINS

In the present collection of organisms which resemble the aerogenes type, 18 at first appeared to occupy an intermediate position where complete correlation could not be established. They persisted in giving methyl red positive and Voges and Proskauer positive reactions in all three of the media employed, after one, three and five days' incubation. Impurity of the cultures was suspected and repeated platings were resorted to. In this way the number of non-correlating organisms was reduced from 18 to 4, although contaminating organisms could not be demonstrated.

The methyl red positive strains whose hydrogen ion concentration was on the border line of the methyl red range (5.7 to 5.9) were made to return to their typical methyl red negative reactions by repeated plating, their pH values being raised to

6.2–6.5. This would indicate that variation within certain limits in the hydrogen ion concentration is to be expected. After 39 replatings the above-mentioned 4 strains still failed to correlate, however, in so far as the methyl red test and the Voges and Proskauer reactions are concerned, both being positive. It is unfortunate that we were unable at the time to make exact gas determinations on these 4 strains, since Clark and Lubs have claimed that "when a perfectly clear-cut correlation fails, there is generally found some abnormality in gas-production. . . ."

All of the 4 peculiar strains gave a pronounced Voges and Proskauer test and were able to attack the purin ring in uric acid; hence, in so far as these two reactions are concerned these organisms resembled typical *B. aerogenes*.

Rogers and his associates reported that the majority of their atypical strains gave a pH value ranging from 5.6 to 6.0, and that most of them (11 out of 16) were Voges and Proskauer positive. Repeated replating might have materially reduced their number of atypical strains, as the pH values given by them were so near the border line of the methyl red indicator range, and as a small variation in the hydrogen ion concentration must be expected of all bacteria.

CORRELATION OF CHARACTERS

Chart VIII presents graphically the results obtained in the study of the various characters of the 447 aerogenes and 173 fecal strains of coliform bacteria. The tests embraced the methyl red, uric acid and Voges and Proskauer reactions, deportment towards glucose, lactose, sucrose and adonitol, indol formation and gelatin liquefaction. As a matter of brevity, the results obtained with the 20 coli strains from soil and the 4 atypical strains are not presented here in chart form. They differ little from the above, aside from a difference in indol production and adonitol fermentation, and the points already mentioned with reference to the methyl red, uric acid and Voges and Proskauer.

It will be observed in the accompanying chart that perfect correlation, exists for both the aerogenes (447) and the fecal coli

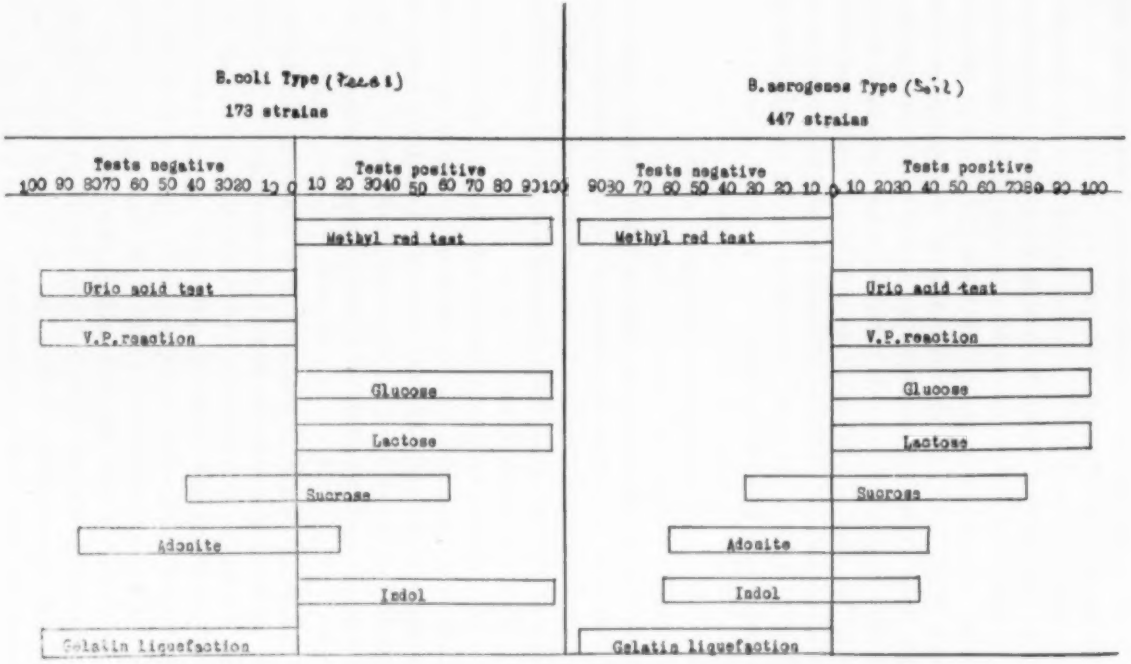


CHART VIII. CORRELATION OF CHARACTERS OF B. COLI AND B. AEROGENES TYPES

(173) types, aside from the reactions in sucrose and adonitol, and indol production of the aerogenes type.

A complete study of gas production in the Durham fermentation tube was made of all of these organisms, both as to volume and gas ratio, in so far as the method would permit, 40 per cent being the arbitrary limit placed for the coli type, and above 40 per cent being taken as an indication of the aerogenes type. Though very crude, this method permitted us to assume from the results obtained a complete correlation between gas volume, gas ratio, the methyl red, uric acid and the V and P reactions. However, on account of the very faulty method employed in the quantitative study of gas production these results are not included in the charts or in any of the tables.

GENERAL DISCUSSION

A survey of the present investigation leads to the conclusion that there exist in nature two distinct types within the colon group of bacteria. These two types, now generally designated as *B. coli* and *B. aerogenes*, can conveniently be set apart from each other by the newer tests.

The direct plating method is better adapted for the isolation of the soil organisms than the combined preliminary enrichment and subsequent plating procedure, because it permits of the study of numerical relationships between bacterial types and their habitat and of the determination of the relative numbers of each type.

The failure to isolate *B. aerogenes* from the feces of man and animals in the present study should not be taken as evidence that this organism is absent from the intestine, but rather as a result of circumstances which permitted its being overlooked. This apparent absence or scarcity is not at all surprising, as Rogers and his associates (1914) found only one high ratio strain in 150 coli-like organisms isolated from bovine feces.

Nothing of importance could be gained through the morphological study of this group of bacteria, since both types present practically the same microscopic picture. The indol test is of

little value, also, and should be used with caution. While all of the *B. coli* strains produce indol, the percentage of positive tests with the aerogenes type is too large to make the test a practical one. The Ehrlich method of determining indol formation is decidedly more delicate than the Salkowski sulphuric acid test, and must be regarded as the more reliable. In the present investigation gelatin liquefaction has been of no value as a test.

The constancy of the hydrogen ion concentration in cultures of the *B. coli* type, as determined by the colorimetric method, is proved beyond doubt by the present study. The hydrogen ion concentration can be adequately measured for practical purposes by the methyl red indicator, as Clark and Lubs have claimed. The synthetic medium of Clark and Lubs appears to be best suited for the colorimetric determination of acidity. Witte's peptone-phosphate-glucose medium of Clark and Lubs answers the purpose well for which it is intended provided that it is kept colorless or practically free from color during the process of sterilization.

Owing to the interference of simultaneous alkali production the limiting hydrogen ion concentration of the aerogenes strains could not be so easily determined. The results of the present investigation show that the hydrogen ion concentration of these strains is completely masked after five days' incubation both in the synthetic and in the Witte peptone-phosphate-glucose medium.

A new set of experiments was conducted to determine daily the hydrogen ion concentration of a collection of 60 aerogenes strains in the synthetic medium for a period of three weeks, in order to ascertain the degree of OH-ion concentration reached by the cultures. The H-ion concentration began to decrease at the end of three days of incubation, and the pH value of the OH-ion concentration proceeded to increase steadily and progressively until the end of the experiment. The greatest H-ion concentration was 4.7, and the highest OH 7.4. It must be apparent, therefore, that length of incubation and temperature are two very important factors in the interpretation of results. If the colorimetric determination is made too soon, or the incu-

bation temperature too low, colorimetric determinations with the methyl red indicator may be positive and correlation with the other tests will be lacking.

Difco peptone-phosphate-glucose medium is less well adapted for the study of hydrogen ion concentration of the aerogenes type than the synthetic and the Witte peptone media of Clark and Lubs, and a greater proportion of failures to correlate must be expected, expecially if the exacting conditions as to length of incubation and incubation temperature are not supplied. The difference appears, however, to be chiefly one of degree rather than kind.

The Voges and Proskauer reaction has proven itself in the present investigation to be of very great value in differentiating between the coli and aerogenes types of bacteria. Neither the character of the medium nor the period of incubation seems to interfere with the carbinol formation. A colorless medium is as important, however, for this test as for the colorimetric determination of the hydrogen ion concentration, owing to the fact that, while the characteristic coloration of the tubes cannot be mistaken, it may be at times obscured by coloring matter in the medium itself, especially if the reaction is relatively weak. Furthermore, intelligent execution of the test is absolutely necessary.

The Voges and Proskauer reaction should be observed within shorter incubation periods than have been customary. A weak reaction persists only for a short time, while a strong reaction may last for several days, with a gradual fading away of the color. A period of from two to eight hours may be regarded as sufficient for a positive V and P reaction, if correctly carried out. Abundant oxygenation of the medium after the addition of the alkali, and proper incubation are important factors.

The uric acid test of Koser constitutes another reliable correlation test. Great care must be exercised, however, in the employment of test tubes, etc. which are free from adhering nitrogenous matter. Koser obtained excellent results with uric acid and hypoxanthine. In the present work xanthine, theobromine and caffeine were employed besides uric acid, the xanthine and uric acid giving very gratifying results. No growth could

be obtained, however, in the theobromine and caffeine synthetic media, either of the coli or of the aerogenes type. This was due, in all probability, to an inhibitive or antiseptic action of these substances, even in the small amount in which they were used. *B. aerogenes* is able to attack the purin ring of xanthine, hypoxanthine and uric acid, and thus appropriate nitrogen in a sufficient degree to develop in the synthetic medium more or less luxuriantly, whereas *B. coli* lacks this property. The differentiation is sharp.

SUMMARY

The present study of coli-like organism in soils of known sanitary quality has shown the great predominance of the aerogenes-cloacae type. Of 467 strains of bacteria isolated from various soils 430 were identified as *B. aerogenes*, 17 as *B. cloacae*, and only 20 as *B. coli*. Furthermore, the sources of the coli strains were shown by the sanitary survey to be not entirely free from animal pollution. All of the 173 organisms found in the feces of 7 men, 2 monkeys and 14 domestic animals were typical *B. coli*. It is apparent from these observations that there is a definite correlation between these types of bacteria and their origin.

An almost perfect correlation could be established by the methyl red, Voges and Proskauer and the uric acid tests.

The limiting hydrogen ion concentration of the coli cultures, as determined by the colorimetric method, varied from pH 4.5 to 5.6 in the synthetic medium, and from 4.6 to 5.8 in the Witte peptone-phosphate-glucose medium. The final hydrogen ion concentration of the cloacae-aerogenes type could not be accurately determined on account of the simultaneous acid and alkali production. The pH value obtained under similar conditions ranged from 6.0 to 7.4 in the Witte peptone medium, and from 6.0 to 6.8 in the synthetic medium.

The respective hydrogen ion concentrations of the colon and aerogenes types of bacteria may be adequately determined for practical purposes by methyl red as an indicator, provided the neutral tint reactions are compared with the reactions obtained by brom cresol purple or some other sharp indicator as a check.

The Voges and Proskauer method of distinguishing between *B. coli* and *B. aerogenes* has proven even more satisfactory than the methyl red test, in that it is simple in operation, and when correctly carried out thoroughly constant in its results. When used with precaution, the uric acid test also is of fundamental importance in differentiating fecal coli from the soil aerogenes type of bacteria.

No definite correlation could be established by means of the indol test. Neither did motility study prove to be of practical value.

Adonitol fermentation did not prove itself to be a satisfactory method of differentiating fecal from non-fecal strains of *B. aerogenes*.

REFERENCES

ARCHIBALD, R. G. 1907 Lactose-fermenting bacilli in surface waters, feces, etc., Wellcome Tropical Research Laboratories, 4th Report, 319.

AYERS, S. H., AND RUPP, P. 1918 Simultaneous acid and alkaline bacterial fermentations from dextrose and the salts of organic acids respectively. J. Inf. Dis., **23**, 188–216.

BARBER, M. A. 1907 On heredity in certain microörganisms. The Kansas Univ. Sci. Bul., **4**.

BERGEY, D. H., AND DEEHAN 1908 The colon-aerogenes group of bacteria. J. Med. Res., **19**, 175–200.

BETTENCOURT, A., AND BORGES, I. Recherches sur le B. coli, des vertébrés inferieurs et des cereales. Archivos do Real Instituto Bacteriologico Camara Pestana, Tome II, Fasc. II, 221. (Cited.)

BOOKER, W. D. 1891 A study of some of the bacteria found in the feces of infants affected with summer diarrhea. Centralbl. f. Bakt., **10**, 284–285.

BROWNE, W. W. 1914 Production of acid by the Bacillus coli group. J. Inf. Dis., **15**, 580–604.

BUNKER, G. C., TUCKER, E. J., AND GREEN, H. W. 1918 A modification of the technique of the Voges and Proskauer Reaction. J. Bact., **3**, 493–498.

BURTON, L. V., AND RETTGER, L. F. 1917 Correlations in the colon-aerogenes group. J. Inf. Dis., **21**, 162–195.

CLARK, W. M. 1915 The final hydrogen ion concentration of B. coli. J. Biol. Chem., **22**, 87–98.

CLARK, W. M. 1915 The reaction of bacteriologic culture media. J. Inf. Dis., **17**, 109–136.

CLARK, W. M. 1915 The final hydrogen ion concentration of cultures of B. coli. Science, N. S., **42**, 71.

CLARK, W. M., AND LUBS, H. A. 1915 The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J. Inf. Dis., **17**, 160–173.

CLARK, W. M., AND LUBS, H. A. 1916 Hydrogen electrode potentials of phthalate, phosphate and borate buffer mixtures. J. Biol. Chem., **25**, 479–510.

CLARK, W. M., AND LUBS, H. A. 1917 Improved chemical methods for differentiating bacteria of the coli-aerogenes family. J. Biol. Chem., **30**, 209–234.

CLARK, W. M., AND LUBS, H. A. 1917 The colorimetric determination of hydrogen ion concentration and its application in bacteriology. J. Bact., **2**, 1–34.

CLEMESHA, W. W. 1912 The bacteriology of surface waters in the tropics. J. Hyg., **12**, 463–478.

DÜGGELI, M. 1904 Die Bakterien-flora gesunder Samen und daraus gezogener Keimpflänschen. Centralbl. f. Bakt., **12**, 602–614.

DURHAM, H. E. 1898 A simple method for demonstrating the production of gas by bacteria. Brit. Med. Jour., **1**, 1387.

EHRLICH, P. 1901 Ueber die Dimethyl-Amidobenzaldehyd Reaction. Berl. Med. Wochenschr., **1**, 151–153.

ESCHERICH, T. 1885 Die Darmbakterien des Neugeborenen und Säuglings. Fortschr. d. Med., **3**, 515 and 547.

EYRE, J. W. H. 1904 On the distribution of B. coli in nature. Lancet, **1**, 548.

FERREIRA, A., HORTA, A. C., AND PAREDES, C. 1908 Recherches sur le B. coli communis de l'intestin de l'homme. Archivos do Real Instituto Bacteriologico Camara Pestana, Tome II, Fasc. II, 153. (Cited.)

FORD, W. W. 1901 Classification of intestinal bacteria. J. Med. Res., **6**, 211–219.

GREENFIELD, MYRTLE 1916 The presence of soil and fecal strains of organisms of the colon-aerogenes group in the waters of Kansas. J. Inf. Dis., **19**, 647–651.

HAMMERL, H. 1897 Die Bakterien der menschlichen Faeces nach Aufnahme von vegetabilischer und gemischter Nahrung. Centralbl. f. Bakt., Ref., 1 Abt., **22**, 706–707.

HARDEN, A. 1901 The chemical action of Bacillus coli communis and similar organisms on carbohydrates and allied compounds. J. Chem. Soc., **79**, 610.

HARDEN, A. 1905 The chemical action on glycose of the lactose-fermenting organisms of feces. J. Hyg., **5**, 488–493.

HARDEN, A. 1905–1906 On Voges and Proskauer's reaction for certain bacteria. Proc. Roy. Soc., Series B, **79**, 424.

HARDEN, A., AND WALPOLE, G. S. 1906 Chemical action of B. lactis aerogenes on glucose and mannitol: Production of 2: 3 butylene-glycol and acetyl-methyl-carbinol. Proc. Roy. Soc., Series B, **77**, 399.

HARDEN, A., AND NORRIS, D. 1911 The diacetyl reaction for proteins. J. Physiol., **42**, 332–336.

HARDEN, A., AND NORRIS, D. 1911–1912 The bacterial production of acetyl methyl carbinol and 2:3 butylene-glycol from various substances. Proc. Roy. Soc., Ser. B, **84**, 492, idem., 1912, **85**, 73.

HELLSTRÖM 1901 Utersuchungen über Veränderungen in der Bakterienzahl der Faeces bei Neugeborenen. Centralbl. f. Bakt., **30**, 309–310.

HOUSTON, A. C. 1897–1898 Chemical and bacteriological examination of soils with reference to amount and nature of organic matter and number and character of bacteria contained in them. Suppl. 27th Ann. Rep. of Loc. Gov. Bd., containing the Rep. of the Med. Officer, p. 251.

HOUSTON, A. C. 1911 Seventh report on research work, Metropolitan Water Board, London.

HOUSTÖN, A. C. 1913–1914 Note on the B. coli test. J. Hyg., **13**, 393–402.

JACKSON, D. C. 1911 Classification of the B. coli group. J. Inf. Dis., **8**, 241–249.

JOHNSON, B. R. AND LEVINE, MAX 1917 Characteristics of Coli-like microorganisms from the soil. J. Bact., **2**, 379–401.

KEYES, F. G. 1909 The gas production of B. coli. J. Med. Res., **21**, 69–82.

KLEIN, E., AND HOUSTON, A. C. 1899–1900 On bacterioscopic analysis of cereals and foodstuffs. Suppl. 29th Ann. Rep. Loc. Gov. Bd., with Rep. of Med. Officer, p. 593.

KLIGLER, I. J. 1914 Indol production in the Colon.-Typhoid group. J. Inf. Dis., **14**, 81–86.

KLIGLER, I. J. 1914 Studies on classification of the Colon group. Idem, **15**, 187–204.

KOSER, S. A. 1918 The employment of uric acid synthetic medium for the differentiation of B. Coli and B. aerogenes. Idem., **23**, 377–379.

KOSER, S. A., AND RETTGER, L. F. 1919 The utilization of nitrogenous compounds of definite chemical composition. Idem., **24**, 301–321.

KONRICH 1910 Zur Bewertung des Bacterium coli im Wasser. Klin. Jahrb., **23**, 1.

LAURENT 1899 Les maladies des plantes. Annal. de l'Inst. Pasteur, **13**, 1–48.

LEVINE, M. 1916 On the significance of the Voges and Proskauer reaction. J. Bact., **1**, 153–164.

LEVINE, M. 1916 The correlation of the Voges-Proskauer and methyl red reactions in the Colon-Aerogenes group of bacteria. J. Inf. Dis., **18**, 358–367.

LEVINE, M. 1916 Acid production and other characters of Bacillus-coli-like bacteria from feces and sewage. Idem., **19**, 773–805.

LEVINE, M. 1918 A statistical classification of the Colon-Cloacae group. J. Bact., **3**, 253–276.

LEVINE, M., WELDIN AND JOHNSON 1917 The Voges-Proskauer reaction and correlated reactions of Coli-like organisms. J. Inf. Dis., **21**, 39–46.

LONGLEY, F. F., AND BATON, W. U. C. 1907 Notes on the determination of B. coli in water. J. Inf. Dis., **4**, 397–416.

MARSHALL, W. E. 1907 The paradimethyl amido benzaldehyde test for indol. J. Hyg., **7**, 581–588.

MCCONKEY, A. 1905 Lactose-fermenting bacteria in feces. Idem., **5**, 333–379.

McConkey, A. 1909 Further observations on the differentiation of lactose-fermenting bacilli, with special reference to those of intestinal origin. Idem., **9**, 86–103.

McFarland, I. 1907 The nephelometer. J. Am. Med. Assoc., **49**, 1176.

Neumann, G. 1910 Der Nachweis des Bacterium coli in der Ausserwelt, besonders auf Nahrungsmitteln. Deut. med. Wochschr., **36**, 2046.

Metcalf, H. 1905 Organisms on the surface of grains, with special reference to B. coli. Science, **22**, 439.

Michaelis, L., and Morcora, F. 1912 Zeitschr. f. Immunitätsforsch., Orig., **14**, 170. (Cited.)

Murray, T. J. 1916 A comparative study of colon bacilli isolated from horse, cow and man. J. Inf. Dis., **19**, 161–174.

Papasotiriu, J. 1901 Untersuchungen über das Vorkommen des B. coli in Teig, Mehl und Getreide, nebst einigen Bemerkungen über die Bedeutung des B. coli als Indikator für Verunreinigung von Wasser mit Fäkalien. Arch. f. Hyg., **41**, 204–210.

Prescott, S. C. 1902 On the apparent identity of the cultural reactions of B. coli communis and certain lactic bacteria. Science, **15**, 363.

Prescott, S. C. 1906 The occurrence of organisms of sanitary and other significance on grains. Biol. Studies by Pupils of W. T. Sedgwick, Boston.

Robinson, H. C., and Rettger, L. F. 1918 The growth of bacteria in protein-free enzyme- and acid-digestion products. J. Bact., **3**, 209–229.

Rogers, L. A., Clark and Davis 1914 The colon group of bacteria. J.. Inf. Dis., **14**, 411–475.

Rogers, L. A., Clark, W. M., and Evans, A. 1914 The characteristics of bacteria of the colon type found in bovine feces. Idem., **15**, 100–123.

Rogers, L. A., Clark, W. M., and Evans, A. 1915 The characteristics of bacteria of the colon type occurring on grains. Idem., **17**, 137–159.

Rogers, L. A. 1916 The type of colon bacillus occurring in surface waters. J. Bact., **1**, 82.

Rogers, L. A. 1918 The occurrence of different types of the colon-aerogenes group in water. Idem., **3**, 313–328.

Rogers, L. A., Clark, W. M., and Lubs, H. A. 1918 The characteristics of bacteria of the colon type occurring in human feces. **3**, 231–252.

Smith, Th. 1868–1893 The fermentation tube with special reference to anaerobiosis and gas production among bacteria. The Wilder Quarter-Century Book, p. 187.

Smith, Th. 1890 Das Gährungskölbchen in der Bakteriologie. Centralbl. f. Bakt., **7**, 502–506.

Sörensen, S. P. L. 1908 Enzymstudien. Biochem. Zeitschr., **7**, 45–101.

Stovall, W. D. and Nichols, M. S. 1918 The methyl red and Voges-Proskauer reactions with special reference to routine water analysis. J. Inf. Dis., **23**, 229–239.

Thompson, J. 1912–1913 The chemical action of B. cloacae on glucose and mannitol. Proc. Roy. Soc., B., **84**, 501.

Voges, O. and Proskauer, B. 1898 Beitrag zur Ernahrungs-physiologie und zur Differential Diagnose der Bakterien der hämorrhagischen Septicämie. Zeitschr. f. Hyg., **28**, 20.

WALPOLE, S. G. 1910-1911 The action of B. lactis aerogenes on glucose and mannitol. Proc. Roy. Soc., B., **83**, 272.

WEST, F. D. 1909 Notes on the Voges-Proskauer reaction for Bacillus coli communis. Am. J. Pub. Hyg., **19**, 227–230.

WINSLOW, C-E. A., AND WALKER, L. T. 1907 Note on the fermentative reactions of the B. coli group. Science, N. S., **26**, 797.

WINSLOW, C-E. A., AND COHEN, B. 1918 The distribution of B. coli and B. aerogenes in polluted and unpolluted waters. J. Inf. Dis., **23**, 90-101.

NOTE ON THE FLORA OF THE STOMACH

G. E. BURGET

From the Department of Hygiene and Bacteriology, University of Chicago

Received for publication February 1, 1920

Sarcinae and other organisms of undescribed form were observed by Goodsir (1842) in the fluid periodically ejected from the stomach of a patient. Hasse and Kolliker (1847) also reported finding sarcinae in gastric juice. Frerichs (1849) confirmed these findings in dogs with a gastric fistula, while the dogs were in a weakened condition. Studying extracts from his own stomach during fasting Abelons (1888–89) isolated *Sarcina, B. pyocyaneus, B. lactis-arogenes, B. amylobacter, Vibrio rugula* and other organisms. Hamburger (1890) reported that gastric juice containing free hydrochloric acid was almost always free from living organisms. From material obtained in the early morning from persons suffering from indigestion, Oppler (1894) found sarcinae in abundance. Kaufmann (1895) in a case of chronic dyspepsia isolated yellow sarcinae, *Micrococcus aurantiacus, Staphylococcus cereus-albus, Bacillus subtilis, Bacillus ramosus,* a large thick bacillus and a short bacillus resembling *B. coli.* One yeast was found in this investigation.

Herter (1907) states that gastric juice in normal abundance as found after a meal acts as a check on the growth to many, and is partially destructive to most, varieties of bacteria. He says that the proteolytic action of pepsin plays a part in this destruction. Gregersen (1916) has shown that gastric juice containing free hydrochloric acid is strongly bactericidal and that the presence of pepsin or combined acid is of no importance in this destruction. Furthermore the presence of bread increased the bactericidal power of the gastric juice to three or four times that of a similar strength of the pure acid in water. Kendall states that certain aciduric bacteria and yeasts may be found occasionally in the normal stomach.

Smithies (quoted from C. H. Mayo, 1914) examined microscopically gastric extracts from some 2400 different individuals with stomach complaint (dyspepsia, indigestion, etc.). He found that irrespective of the degree of acidity of such extracts bacteria were present in 87 per cent of the cases. After cultural studies of the saliva from dyspeptic patients he concludes that pus forming organisms have their growth retarded in gastric juice while bacilli as well as leptothrices thrive in the stomach. The degree of acidity of the extracts is not mentioned.

In investigating the flora of the gastric contents from different individuals, Fowler (1916) found besides bacteria many different yeasts.

The literature on the flora of the stomach has been confined principally to findings that have been made during pathological conditions in the stomach. The degree of acidity and the amount of free HCl present have not always been given due consideration in the determinations.

The individual (Fred V.) from whom the specimens were taken for this investigation has a complete closure of the oesophagus and a gastric fistula. The fistula is of several years standing and does not interfere with his general health which was good at the time this work was carried out. The usual source of contamination from swallowed saliva was eliminated.

Two or three hundred cubic centimeters of sterile water were injected into the stomach three or four hours after a light meal to carry off the residuum. About an hour later a specimen of psychic secretion was taken by aspirating into a sterile bottle. The free and total acidity were determined and plates immediately made. Those specimens only were considered which were clear and of normal acidity, the free acid varying between 0.15 and 0.25 per cent and the total acid between 0.20 and 0.37 per cent. All specimens of normal acidity showed comparatively the same group of organisms present. If the acidity were below normal quite a variety of organisms were contained. On the other hand it seems that relatively few bacteria are able to resist the HCl of gastric juice when in normal concentration.

The count per cubic centimeter of juice from some 20 specimens plated on glucose litmus agar plates shortly after collection varied

between 25,000 and 100,000. With the exception of no. 7, a yeast, these organisms were in relatively small numbers. However this count was reduced more than one half if the specimens were allowed to stand 24 hours at room temperature. The destruction was very marked in all organisms except the one yeast. This yeast not only resisted the acid of the gastric juice but

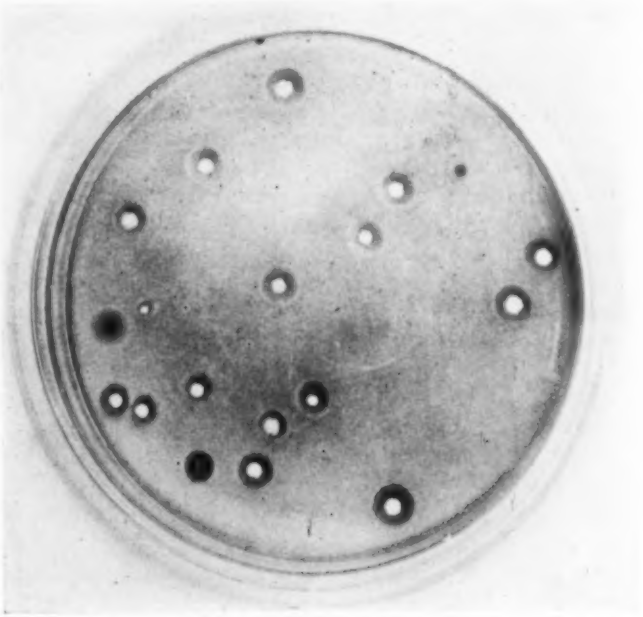


Fig 1. Photograph of a Glucose Agar Plate. This plate was made from a specimen of gastric juice diluted 1–2000 showing predominance of no. 7 by characteristic nail head appearing colonies. Specimen stood for twenty-four hours at room temperature before plating.

thrived in it. Three chromogenic organisms were among those usually found but these seemed to have no greater resistance to the gastric juice than the non-chromogenic forms present.

These findings indicate that the flora of the "empty" stomach of Mr. V. is fairly constant. In clear specimens of normal acidity

the number of different organisms is few and they are unrelated making it impossible to classify them into a definite group or groups. Extracts with acidity below normal have a much higher bacterial content and a greater variety of organisms.

Not being able at the time this work was being carried on to spend further time in the study, a somewhat incomplete description of the following organisms is made necessary:

No. 7. A yeast. Colony on glucose agar, round with rough edges made of projecting filaments; white to gray with raised center giving nail head appearance (see photograph).

Milk. No acid.

Lactose broth: flocculent precipitate after two weeks, no fermentation, no gas, no acid.

Glucose agar stab: no gas.

Glucose litmus agar: Acid formed.

Gelatin: Not liquefied.

No. 4. A yeast. Colony heavy gray on glucose litmus agar, small, acid forming.

Glucose agar slant: Heavy gray waxy growth.

Milk: Acid formed; proteolysis.

Gelatin: Liquifaction.

Lactose broth: Filaments formed; no gas.

Resembles bacterium, has granules and vacuoles; Gram positive.

No. 5. Bacillus.

Colony: Thin, gray and rather large. A short bacillus forming chains.

Glucose litmus agar: No acid.

Glucose stab: No gas.

Litmus milk: Decolorized, no coagulation.

Lactose broth: No gas, no acid, gray precipitate.

Gelatin: Not liquified, (growth at 20 degrees C.).

Non motile, spore forming, Gram positive.

No. 3. Coccus form.

Heavy colony slightly yellow on Russell.

Glucose stab: No gas, no acid.

Lactose broth: Cloudy but not much precipitate, no acid, no gas.

Milk: No acid.

Large cocci with tendency to pairs.

Gram positive.

No. 9. Coccus form.

Colony: White, rough edge made of rounded projections; large.

Grows poorly on glucose agar; grows better on plain agar slightly alkaline.

Milk: No acid until several days, when small amount is noticed.

Large cocci with tendency to bunches.

Gram positive.

No. 10. Sarcina flava.

Colony: Small, yellow, translucent.

Litmus milk: Decolorized, no coagulation.

Gelatin: Not liquified.

In cubes and bunches, not regular even when examined from broth. Lehman and Newman, p. 159; Chester, p. III.

No. 14. Boas Oppler bacillus.

No. 13. A short bacillus with some coccus forms.

Colony: Small, pink on Russell's.

Litmus milk: No acid, no coagulation.

Have not been able to identify.

REFERENCES

ABELONS, J. EMILE 1888–89 Compt. rend. Acad. d. sc. Paris, 1888, **108**: 310–313; Compt. rend. Society de biol. Paris, 1889, **9**: 1,8 6–89; Montpel. & Paris. C. coulet. Lecasnier & Babi, 1889, 163 p.

FOWLER Personal communication.

FRERICHS 1849 Haser's Archiv. f. d. ges. Med. **10**: 137.

GOODSIR, JOHN 1843 Schmidt's Jahrbucher **39**: 305.

GREGERSEN, J. P. 1916 Central. f. Baker., Parasit, and Infek **77**.

HASSE AND KÖLLIKER 1847 Archiv. f. physiol. Hulk, **6**: 762.

HAMBURGER, H. 1890 Central bl. f. klin. Med. Leips. **11**: 425–437.

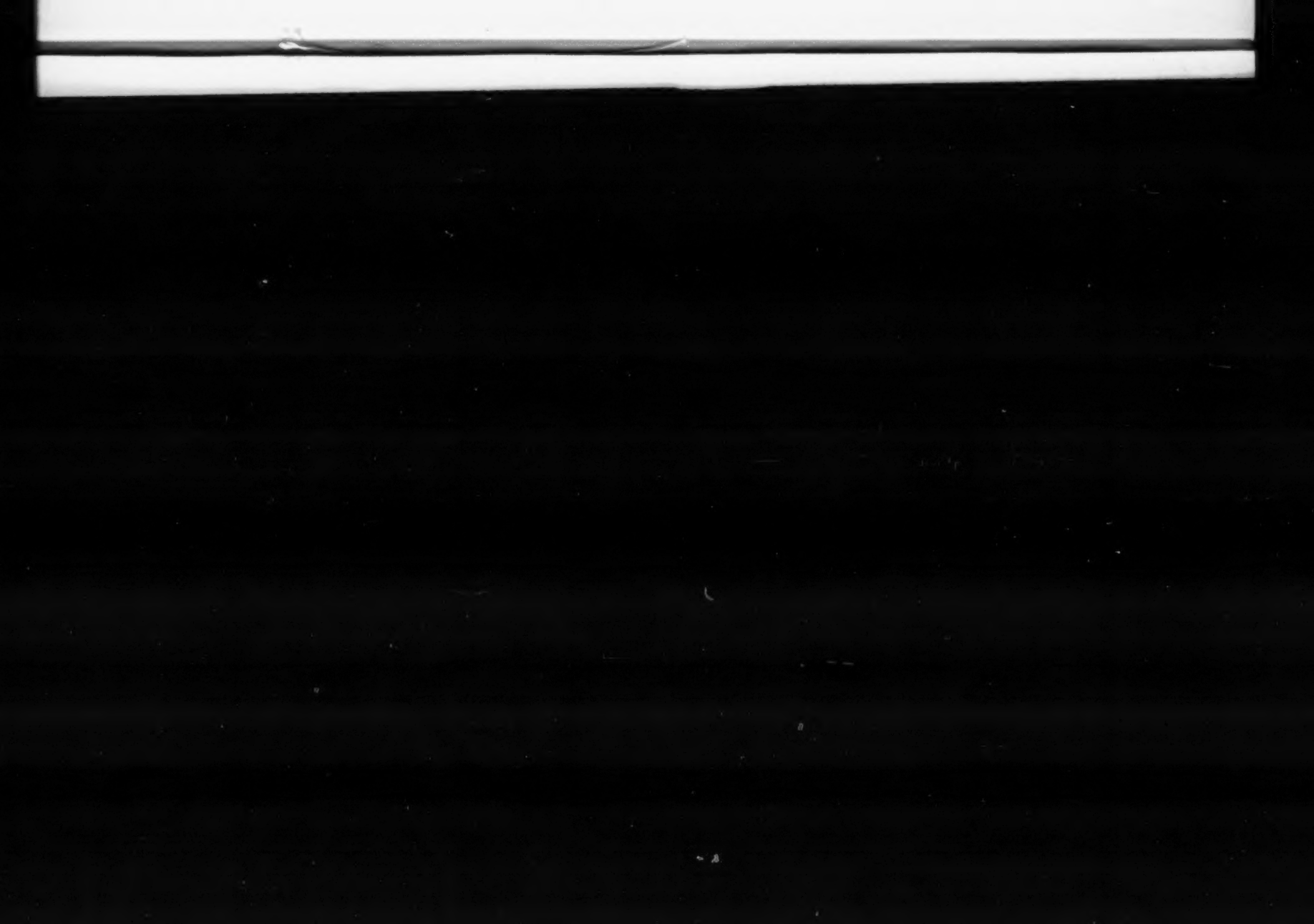
HERTER 1907 Bacterial infection of the digestive tract.

KAUFMANN, J. 1895 Berliner klinische Wochenschrift, **32**: 117 and 147.

KENDALL 1916 Bacteriology.

OPPLER, DR. BRUNO 1894 Munchener Medicinische Wochenschrift, **41**: 570.

SMITHIES 1914 J. A. M. A. **63**: No. 23.



THE RELATIVE EFFECT OF PHOSPHATE-ACETATE AND OF PHOSPHATE-PHTHALATE BUFFER MIXTURES UPON THE GROWTH OF ENDOTHIA PARASITICA ON MALT EXTRACT AND CORN MEAL MEDIA

M. R. MEACHAM, J. H. HOPFIELD AND S. F. ACREE

Received for publication February 27, 1920

As described in another report,[1] a mixture[2] of acetic acid and dipotassium hydrogen phosphate (K_2HPO_4) has been used in increasing the buffer effect of several media. As the writers had observed that some kinds of organisms grow upon rather weak solutions of potassium acid phthalate, it was thought well to try the latter salt instead of acetic acid as a buffer material and to test further its effect upon the growth of *E. parasitica* (Murr.) Anders. for which it was desired to use the buffered media. Although acid potassium phthalate and dipotassium hydrogen phosphate do not give as straight a buffer curve as do the acetic acid and dipotassium hydrogen phosphate, and hence have not been used in general culture work, the following results may be of value.

The media used were 2.5 per cent malt extract agar or corn meal extract agar buffered with M/50 molar K_2HPO_4 plus either M/50 acetic acid or M/50 acid potassium phthalate. The pH values of these media are about 5.7 and do not vary from each other in pH by more than 0.2. This pH is at or near the optimum for the organism, as reported in another paper,[3] but is also

[1] Paper read before the Meeting of the American Chemical Society at Philadelphia, September, 1919, and appearing soon in the Journal of Bacteriology.

[2] We also make the same mixture by using weighed quantities of pure anhydrous sodium or potassium acetate and potassium dihydrogen phosphate in proper proportions. Each salt can be sterilized separately in weighed quantities and the mixture can be made afterwards under sterile conditions.

[3] See an article appearing in the Journal of Bacteriology in March, 1920. An exhaustive report on the work on *Endothia parasitica* will appear in the near future. See Science **48**: 449–450, November 15, 1918.

in the pH range which changes very greatly between the straight parts of the neutralization curves for the first and second hydrogen ions of phosphoric acid. Our central idea in using these *buffer mixtures*[4] is to straighten out these H ion inflection curves and hence to keep the pH near the optimum by permitting only small changes in acidity by the organic acids generated by the fungi or bacteria. Acetic, phthalic, formic, malic, asparaginic, aminoacetic and other acids with ionization constants between the three constants for phosphoric acid, i.e., between 1.2×10^{-2} and 1.1×10^{-7} and 1.1×10^{-7} and 10^{-12}, accomplish the desired end excellently.

Since the co-workers of one of us[5] have shown that the activity of both ions and molecules of acids, bases and salts must be measured in all cases, and that in many reactions the nonionized molecules are even more active than the hydrogen or other ions, we shall in all cases consider that the growth curves of every organism are influenced by every ionized and nonionized substance present. In the present case, then, the better growth in the phosphate-phthalate mixture shows that the phthalate or acid phthalate anion, or the nonionized phthalic acid compounds present, must be considered better for *E. parasitica* than the acetate ion or molecular acetic acid compounds. Since, in general, the molecular forms of stronger acids have greater activities than the molecular forms of weaker acids, it will be interesting to see how stronger acids like phthalic increase (or depress) the rate of growth, or other biological factors, more than does the weaker acetic acid, for example, as in the present study. In such comparative studies the factors known to be of great im-

[4] General formulae for calculating the form of the titration curves and data for mixtures of a large number of acids and bases have been developed. These and data on the growth of organisms on a number of such regulated media will be published soon.

[5] Researches by Nirdlinger, Rogers, Shadinger, Loy, Desha, Chandler, Marshall, Johnson, Harrison, Robertson, Myers, Gruse, Shrader, Taylor, and Brown in cooperation with one of us. See Am. Chem. Jour. **39**:, 275 (1908); **49**: 116 (1913); **43**: 519; **49**: 474; **49**: 177, 369; **49**: 122, 132, 485 (1913); **48**: 374; **49**: 350, 378, 396, 403; **41**: 466. Jour. Am. Chem. Soc. **37**: 1902. Jour. Phys. Chem. **19**: 589 (1915); **20**: 365 (1916).

portance, such as the hydrogen ion concentration, must be kept constant while the unknown factors are made the variables.

EXPERIMENTAL

In the first set of comparisons made by the writers[6] on malt extract regulated with these two buffer mixtures, both media were excellent, but the phosphate-potassium acid phthalate mixture seemed to give the better growth. Mr. Hopfield then very carefully repeated the tests and found the better growth with acid potassium phthalate. In order further to verify the results, Mr. Hopfield ran two other sets of tests, side by side, using the same media given above and also media made up in the same way excepting that corn meal gruel was substituted for the 2.5 per cent of malt extract. The corn meal gruel was prepared by cooking 7.5 grams of yellow corn meal and about 200 cc. of water in a water bath at 60°C. for one hour. After allowing the meal to settle the liquid was decanted and fresh water mixed with the meal. After allowing the meal to settle again the water was decanted and added to the liquid first decanted. This liquid was used in making up 250 cc. of each of the two media after adding the proper amounts of K_2HPO_4 and acetic acid or acid potassium phthalate, and agar.

The average diametric growth, in millimeters, of four Petri dishes of each medium is given below:

Medium	*Diametric growth in mm. in 10 days at 25°C.*
2.5 per cent of malt extract, M/50 K_2HPO_4 and M/50 acetic acid..	64
2.5 per cent of malt extract, M/50 K_2HPO_4 and M/50 acid potassium phthalate.....	80
Corn meal with M/50 K_2HPO_4 and M/50 acetic acid.....	55
Corn meal with M/50 K_2HPO_4 and M/50 potassium acid phthalate	71

Thus it is very evident that the potassium acid phthalate allows more rapid growth of *E. parasitica*, although both media are excellent.

[6] The data given here were obtained during the summer of 1918.

CONCLUSIONS

1. Malt extract and corn meal extract have been buffered with phosphate-acetate mixtures and with phosphate-phthalate mixtures to straighten out the first phosphate inflection curve and regulate the hydrogen ion concentration accurately between pH = 1 and pH = 8. By using asparaginic acid and other amino-acids the acidity can be regulated accurately between pH = 1 and pH = 13.

2. It is shown that *Endothia parasitica* (Murr.) Anders. grows excellently on all of these media when regulated at optimum values close to pH = 5.7, but that the phosphate-phthalate buffer is somewhat (25 per cent) better. Furthermore the malt extract seems to give slightly (10 per cent) better growth than a corresponding corn meal medium containing the same buffers.

3. It is suggested that biologists should study the influence of all chemical species present, such as the activity of the nonionized acids, bases and salts along with the various simple and complex ions.

A METHOD OF DETERMINING THE RELATIVE TOXICITY OF SODIUM, POTASSIUM, LITHIUM, AND OTHER IONS TOWARD ENDOTHIA PARASITICA: DATA ON SODIUM CHLORIDE

M. R. MEACHAM, J. H. HOPFIELD AND S. F. ACREE

Received for publication February 27, 1920

In making a detailed chemical program in coöperation with Dr. Caroline Rumbold and in connection with her investigations[1] on the injection of trees with chemicals to bring about immunity against attack by fungi and bacteria, it was considered important to conduct a laboratory study with culture media on the relative toxicity or effect of the molecules, anions and cations of various sodium, potassium, lithium, calcium and other salts. As the lithium salts which Dr. Rumbold found most effective were not readily available during the War, the present paper deals with the toxicity of sodium chloride.[2] If a constant hydrogen ion concentration near the optimum for *Endothia parasitica* (Murr.) Anders. were used in the media and if the salts were compared on the basis of chemically equivalent quantities with a common anion, it was thought that the tests would show the relative toxicity of both the cations and nonionized molecules of each salt. Knowing the relative concentrations of the cations and nonionized form of a given salt present in decreasing quantity in a series of samples of the medium, it is easy to calculate the activity of the different ionic or molecular species from a series of simultaneous equations having the form (1) which was

[1] The injection of chemicals into chestnut trees. American Journal of Botany 6: December, 1919.

[2] Ferdinand Wolesky stated in Papier Zeitung, 21: 563 (1896) that 0.05 per cent solutions of sodium chloride prevent the growth of organisms on wood pulp. Such a high toxicity for sodium chloride, corresponding to the best creosotes, is not substantiated by the present work in which 75 times as much sodium chloride decreased the growth only to 25 per cent of its normal value.

(1) total activity = cation activity × cation concentration + anion activity × anion concentration + molecular activity × molecular concentration + ...

developed[3] for studying the activities of various ions and molecules in pure chemical reactions. When a given series of salts with a common anion in the same concentration is used, this equation is naturally simplified and gives the values for the specific effects of the cations and molecules.

There are two good ways to vary the cation and molecular species. The first is to use buffer mixtures having common anions but different cations. For example, the mixture of M/50 phosphoric acid and M/50 acetic acid employed as buffer material in the present study can be titrated or neutralized with sodium, potassium, lithium, etc. hydroxides to keep the hydrogen ion concentration at desired values. In such a case the ionization values of these bases and their salts are already nearly identical and the chemical aspects of the problem are much simplified. The second method is simpler in technique though slightly more complicated electrochemically, and involves adding sodium, potassium, lithium, etc., salts with common anion to the medium buffered with the same substances throughout. If the pH of the medium is to be kept absolutely constant while the concentration of one of the salts is to be varied, it is obvious that the salts used must be neutral compounds from strong acids and bases. The chlorides of the metals to be used fulfill this condition as well as any salts. Besides, the chlorides of all these metals except calcium are about equally ionized—another condition to be considered. Even this procedure involves changes in the pH or acidity, because of changing ionization of the buffer salts, but such deviations in pH are known to be small and will be corrected in our future more accurate investigations.

[3] Researches by Nirdlinger, Rogers, Shadinger, Loy, Desha, Chandler, Marshall, Johnson, Harrison, Robertson, Myers, Gruse, Shrader, Taylor, and Brown in cooperation with one of us. See Am. Chem. Jour. **39**: 275 (1908); **49**: 116 (1913); **43**: 519; **49**: 474; **49**: 177, 369; **49**: 122, 132, 485 (1913); **48**: 374; **49**: 350, 378, 396, 403; **41**: 466. Jour. Am. Chem. Soc. **37**: 1902. Jour. Phys. Chem. **19**: 589 (1915) **20**: 365 (1916).

Tests already reported[4] showed that the optimum initial pH for *E. parasitica* is about 5.0. Besides possessing the proper initial pH the medium should have a fairly strong buffer effect in the region between pH = 3 and pH = 7 in order that it may be stable and resist any marked changes in pH which might be brought about through the organic acids generated by the fungus, accidental impurities, carbon dioxide of the air, solubility of glass, etc. Accordingly 2.5 per cent malt extract medium buf-

TABLE I*

To test the toxicity of NaCl *toward E. parasitica; 2.5 per cent malt extract* + M/50 K_2HPO_4 + M/50 *acetic acid* + *1.5 per cent agar; Petri dish cultures*

CONCENTRATION OF NaCl IN MEDIUM	RADIAL GROWTH IN MILLIMETERS IN FIFTEEN DAYS AT 25°C.	CONCENTRATION OF NaCl IN MEDIUM	RADIAL GROWTH IN MILLIMETERS IN FIFTEEN DAYS AT 25°C.
0.0 Normal	†	0.257	26
0.05N Normal	†	0.322	23
0.073N	†	0.363	22
0.0975	†	0.35	24
0.121	39	0.40	20
0.145	38	0.45	20
0.167	35	0.495	19
0.19	35	0.54	12
0.212	28	0.585	13
0.235	25	0.630	15

* Data taken from notes made by Mr. Hopfield.

† Indicates dish completely covered.

fered with M/50 K_2HPO_4 and M/50 acetic acid was chosen. The acetic acid acts as a buffer to smooth out the marked change in pH found in passing from the neutralization of the first hydrogen ion to the second of the phosphoric acid, as discussed in other articles.[5] This medium was brought to a pH of about 5.0 by the addition of 0.5 cc. of normal hydrochloric acid to each 100 cc. To this medium, containing 1.5 per cent agar, was added pure sodium chloride in varying concentrations ranging from 0 to 0.63 normal. Three dishes of each salt concentration were

[4] This Journal, March, 1920. See reference 5, this article.

[5] Address before Amer. Chem. Soc., Philadelphia, September, 1919. Science 48: 449–450, November 15, 1918. This Journal, March 1920, and unpublished articles.

poured and inoculated. Table 1 shows the average radial growth of the culture in fifteen days at 25°C. The curve sheet contains a graph prepared upon the basis of these data, and shows the decrease in growth with increase of salt concentration.

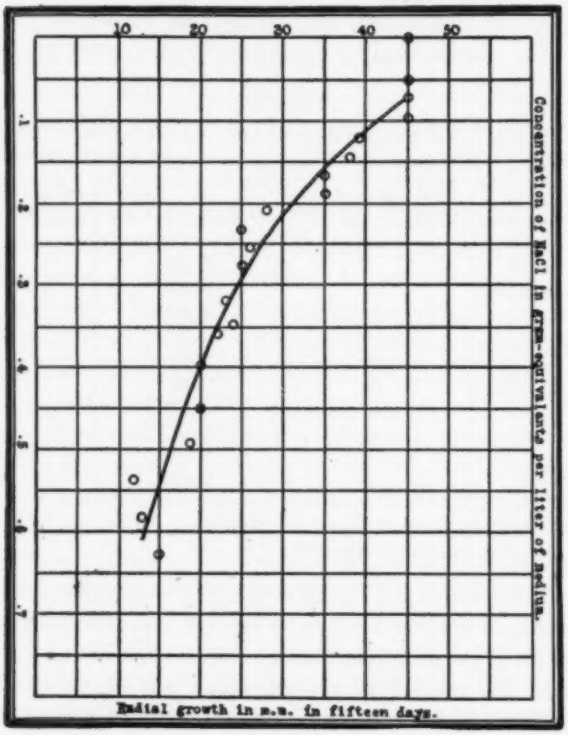


FIG. 1

It is clear from the above table that sodium chloride is only mildly toxic to *E. parasitica* and that solutions as concentrated as 0.63 N, or 3.65 per cent, decrease the growth to possibly 25 per cent of the normal value under the conditions used. Al-

though the points representing radial growth in the graph do not lie exactly on the smooth curve they are sufficiently close to it to show fairly accurately the relation between decreasing growth and increasing sodium chloride concentration. It is clear that this relation is not a straight line but one showing a decreasing ratio (decrease in growth/salt concentration) with increase in sodium chloride. As the ratio (salt ion/salt concentration) also decreases with increasing concentration of sodium chloride, the data may have some weight on the hypothesis that the ions are more active than the salt molecules. This idea should be held with reservations, however, until we can secure data on the other salts by the alternative methods discussed above and learn the specific effects of the various cations, anions and molecules.

CONCLUSION

1. *Endothia parasitica* (Murr.) Anders grows excellently on malt extract, corn meal extract and bean decoction media regulated between pH = 5 and pH = 7, and buffered with M/50 K_2HPO_4 and M/50 acetic acid to prevent the marked changes in pH produced in passing from the neutralization of the first hydrogen ion to the second of phosphoric acid.

2. In such a buffered malt extract medium regulated at about pH = 5, the addition of sodium chloride up to 0.63 N or 3.65 per cent causes a gradual decrease in the rate of growth to about 25 per cent of the normal value. This salt is therefore only mildly toxic, contrary to the statements of Wolesky.

3. The form of the growth curve suggests the possibility that the salt ions are more toxic than the salt molecules, but this conclusion should be considered tentative until extensive work on this and other salts can give the values for the toxicities of the cations, anions and nonionized molecules of such electrolytes.

REPORT OF COMMITTEE ON DESCRIPTIVE CHART

PART II. REPORT OF PROGRESS DURING 1919

H. J. CONN, *Chairman*, H. A. HARDING, I. J. KLIGLER, W. D. FROST, M. J. PRUCHA, AND K. N. ATKINS

Received for publication January 24, 1920

Two years ago this committee recommended to the Society a new descriptive chart in the form of a folder. The folder was intended primarily for use in instruction, although it was felt that it might be satisfactory in some forms of research work. When first brought to the attention of the Society, this chart provoked much discussion, and it was finally decided that the only way to determine its value would be to print it and test it by use in a practical way. Hence the chart was not officially adopted by the Society, but the committee was directed to have it printed and to distribute it. At the present time the committee does not think it necessary to ask for official adoption of this chart. In the first place, the orders that are constantly being received for it, many of them repeat orders, have endorsed it sufficiently; and in the second place, we hope to draw up a new chart during 1920 embodying some of the suggestions that have been made while the folder has been in use. During the half of 1918 in which the folders were on sale, 10,000 were sold, and during the last year 8000—a total of 18,000. Meanwhile during these same eighteen months, only 4400 of the old charts have been called for.

The extent to which the new chart has been purchased shows that it is generally preferred to the old single page chart. This is natural, for the greatest use of the chart is made in teaching laboratories, and the folder was designed primarily for instruction. It is still in need of revision, however. Besides various matters of detail that need changing, three fundamental ques-

tions have been raised: (1) Should the chart be a folder or a single sheet (i.e., two pages or four)? (2) Shall the use of the group number be continued? (3) Shall a special space be devoted to pathogenesis? These three questions require some discussion.

TWO PAGES VERSUS FOUR PAGES

The committee has never been unanimously in favor of a four page folder. In fact some of us have always been of two minds on the subject. But enquiries made a few years ago seemed to show that nearly all the institutions then printing charts of their own for instruction purposes were using a folder. Accordingly the committee decided in favor of a folder for the instruction chart, thinking that the old card still remained to be used by those who preferred the single sheet. The old card, however, is out-of-date and calls for much unneeded information, and hence does not fill the need for a single sheet chart.

It seems, therefore, as if it would be well to replace the old card of 1914 with a new chart which would be a compromise between the two forms now in use. The experience of the last two years has shown that even research men prefer a chart considerably simpler than the old card and are using the folder to some extent, although they do not like its bulk. Hence it is not impossible that a compromise form might prove more satisfactory both to teachers and to research men than either of our present charts. It should be a single sheet of quarto size, printed on both sides, with the most important information on its face, calling for more information than the folder, but less than the old card, more concise than the folder but with more blank space for sketches and notes than allowed by the old card. If both the new chart and the folder were then put on sale, a couple of years experience would show whether there would be call for both forms or if one could supplant the other.

During the coming year the committee plans to take this matter up with all the institutions that have recently ordered our charts, and to ask the instructors whether they prefer a folder

or a one-page chart. All bacteriological instructors, therefore, who read this report, if using the charts or interested in them, are urged to communicate with the committee chairman[1] and indicate their preferences in this matter. With such information at its disposal, the committee will be able to inform the Society as to what kind of a chart is generally desired, that the Society can act intelligently in deciding whether to approve a revised chart.

THE QUESTION OF THE GROUP NUMBER

The present group number is the weakest part of the chart. It is still there in its present form simply because of the unwisdom of changing it too often and because the committee has never been able to decide what it preferred in its place. As to its value, every opinion is held among the members of the committee: from the conviction that the group number is the most important part of the chart to the feeling that it should be omitted entirely. A compromise judgment is about as follows: that the present group number may be unhesitatingly condemned; that *a* group number, calling for the right information and properly used, might be very valuable; that revision of the present form must be delayed no longer and that the new group number must be in such a form as to prevent confusion with the old one.

One reason for the disrepute into which the group number has fallen is that too much has been expected of it. By some it has been assumed that it could replace the species name, an idea not apparently held by the men who first proposed the group number. It should be regarded merely as the simplest form of concise description of an organism—a sort of short-hand notation for recording the salient characteristics. Its chief value is as an index

[1] At the meeting of the Society it was decided not to continue the committee on the chart, as such, but to appoint a new committee on bacteriological technic. This new committee is to have a somewhat changeable membership, in order that it may be composed of men doing actual work on problems of technic. Its present members are: H. J. Conn (Geneva, N. Y.), chairman, I. J. Kligler, K. N. Atkins, J. F. Norton, and G. E. Harmon. This committee is to continue the work on the chart as a part of its program.

number to be used when the charts are filed according to the characteristics of the organisms.

To avoid further misconception and to emphasize this particular use of the number, the present plan is to call the new form an index number instead of a group number; to drop the generic symbol before it and to put it in such a form that confusion with the old group number will be impossible; and to see that all the information called for in the index number is similarly recorded elsewhere on the chart so that the description will be complete if the student decides not to use the index number. By doing this it is hoped to meet all the criticism that has been directed against the group number.

SPACE FOR RECORDING PATHOGENICITY

Various cards have been devised with space for recording the pathogenicity of an organism. Of these the best have merely the heading pathogenicity followed by a blank space. This feature can very easily be included on the new chart, although this space would be of no use in the case of saprophytic organisms. On the other hand, non-pathogenic organisms generally must be investigated by special tests for which blank space would be desired; and it seems a waste to leave separate blank spaces for both sets of tests. For this reason, the folder has an entire page blank for notes, the idea being that it might be used to record pathogenicity or the special tests made for non-pathogenic organisms. As this use of the space does not seem to be understood by all users of the chart, it is proposed on the revised chart to have a space headed something like this: "Special Reactions and Environmental Relationships (e.g., Pathogenesis)."

METHODS

When the 1914 card was prepared, the glossary and notes were omitted from the back of it on the understanding that a pamphlet was to be drawn up by the committee giving a glossary of terms used on the chart and methods to be employed in making the tests. After some unavoidable delay this pamphlet was finally

prepared and was printed in the Journal of Bacteriology as the report of this committee for 1917. One copy has been furnished with every order of 100 or more charts, and additional copies have been on sale at about cost price. More detailed information as to the acid and nitrate tests was given in the 1918 report, of which, also, copies are on sale. The edition of the 1917 report is not so nearly exhausted that it has been reprinted in slightly revised form as Part I of this report in the last number of this Journal.

Among the special investigations on methods which the committee has undertaken is a study of the Gram stain, and the following paper is the result. This modification of the Gram stain looks very promising because of the stability of the solutions. The committee is recommending it for provisional use, but is not endorsing it until it has been more thoroughly tested. Of the more commonly used methods, the Stirling technic[2] seems to give the most satisfactory results, and can be safely recommended for general use until the value of this more recent technic has been determined by practical use.

[2] Method 1 described in Committee Report for 1918, J. Bact. **4**, 113.

REPORT OF COMMITTEE ON DESCRIPTIVE CHART

PART III. A MODIFICATION OF THE GRAM STAIN

KENNETH N. ATKINS

Dartmouth Medical School

The aniline gentian violet staining solution of the Gram stain is not stable, usually becoming obviously decomposed in a few weeks. One is occasionally apt to use a stain which is too old to give accurate results. The diversity of procedure in using this stain, as noted in textbooks of bacteriology, is doubtless an outgrowth of attempts to make the stain more useful and consistent in its action. The use of a stable staining solution would tend towards uniformity of procedure and accuracy of results. The writer does not wish to introduce a new stain, to be used in place of the Gram stain (Gram, 1884), but rather, to modify that well known stain so that it will be stable while its chemical properties remain unchanged.

The stain is as follows:

Staining solution

Saturated 95 per cent alcohol solution gentian violet	1 part
Aniline sulphate, 1 per cent aqueous solution	3 parts

Iodine solution

Iodine	2 grams
Normal solution sodium hydroxide	10 cc.
Water	90 cc.

(Dissolve the iodine in the sodium hydroxide solution and add the water.)

Decolorizing solution

Alcohol	95 per cent

Technique

The slide is prepared as usual and stained for one minute. Wash briefly to remove the excess of stain and apply iodine solution for one minute.

Wash thoroughly before decolorizing in alcohol for two minutes or until the alcohol is no longer colored. In thick smears, as pus, the decolorization may be continued for ten minutes.

Wash. Counterstain. Wash, dry and mount.

In this proposed modification aniline sulphate is used in place of aniline and sodium hydroxide is added to the iodine solution. Otherwise there is no change in the solutions. Aniline sulphate is a stable salt, both in its dry form and in solution. The aniline-sulphate-gentian-violet stain should keep indefinitely. The instant that the modified iodine solution is placed on the slide preparation, stained with aniline-sulphate-gentian-violet, the aniline sulphate is decomposed and free aniline liberated. The effect then, is the staining of the bacteria according to Gram's classical method with a solution that is prepared as used.

The addition of a weak solution of sodium hydroxide to iodine causes the formation of sodium-hypoiodite. In the solution above, the amount of sodium hydroxide is not sufficient to convert all of the iodine to sodium-hypoiodite and leaves an excess of iodine in solution. The amount of free iodine present is about the same as that present in the iodine solution of the Gram stain. This solution contains sufficient hydroxyl-ion concentration to set free aniline from aniline sulphate.

Other aniline salts were tried, such as aniline acetate, oxalate, nitrate and hydrochloride. The aniline acetate was discarded because of its instability. Difficulty was experienced in the use of aniline nitrate and aniline hydrochloride. The bacteria did not take the color of the gentian violet properly and the finished preparation was decidedly lacking in brilliancy. Apparently there was a decided difference in the penetrating power (if the term may be properly used), of the aniline salts. Aniline-oxalate-gentian-violet seemed to penetrate, i.e., stain the bacterial cells in the primary step of the Gram stain, fully as well as aniline-gentian-violet. Aniline-sulphate-gentian-violet also penetrated well but specimens stained with aniline hydrochloride and aniline-nitrate-gentian-violet and washed, dried and examined before the addition of the iodine solution, appeared a light purple color instead of a dark purple. This suggests the testing

of the more uncommon aniline salts with the hope that one may be found which will be entirely stable and have great penetrating power.

As an alternative method, aniline oxalate may be used in place of aniline sulphate in this modified stain. This salt seems to have slightly better penetrating power than the aniline sulphate and has the advantage that staining may be accomplished in thirty seconds instead of one minute. This stain was kept for six weeks in the sunlight out doors and in the incubator for two months and no change could be detected in its staining qualities. It is thought, however, that the aniline oxalate stain would not be as stable as the aniline sulphate stain because aniline oxalate very slowly decomposes in solution, liberating free aniline, while aniline sulphate solution is stable.

The aniline-sulphate-gentian-violet stain as here outlined was tried on various kinds of bacteria and in all cases the results were similar to those obtained with the Gram stain. Control slides of Gram positive organisms made according to the usual Gram method, showed the bacteria slightly less densely stained by this modified method. This difference was no greater than the difference detected in a comparison of slides made with American and Gruebler dye-stuff in the customary manner. Gram negative bacteria with this modified stain seem to be more nearly colorless than the controls made by the Gram method. The degree of difference between a Gram negative and a Gram positive stain seems to be entirely comparable in the two methods. Different strengths of solutions, imported and domestic dye-stuff and various time elements of staining, washing and decolorizing were tried and there seemed to be a fairly wide range of limits, with the production of apparently the same result in the finished specimen. Two points should be noted; first, the primary washing of the slide after staining with aniline-sulphate-gentian-violet should be brief (a long washing of more than half a minute tending, on account of the solubility of the aniline salt, to reduce the brilliancy of the preparation); second, the modified iodine solution should be left in contact with the slide for one min-

ute. With these two exceptions the times of staining and washing may be considerably increased or decreased with little or no apparent change of result.

The writer has received from Prof. C. E. Bolser, of the Chemistry Department of Dartmouth College, important suggestions in this work and desires to express his indebtedness to him.

REFERENCE

GRAM, C. 1884 Fortschr. d. Med., **2**, 185.